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"To accomplish great things, we must not only act,
but also dream; not only plan, but also believe."

-Anatole France

University of Alberta

**Identification and fine mapping of QTL for
growth traits on bovine chromosomes 2, 6, 14, 19, 21 and 23
within one commercial line of *Bos taurus*.**

by

Jenifer Lee Kneeland



A thesis submitted to the Faculty of Graduate Studies and Research in partial
fulfillment of the requirements for the degree of **Master of Science**

in

Animal Science

Department of Agricultural, Food and Nutritional Science

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University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Identification and fine mapping of QTL for growth traits on bovine chromosomes 2, 6, 14, 19, 21 and 23 within one commercial line of *Bos taurus*** submitted by **Jenifer Lee Kneeland** in partial fulfillment of the requirements for the degree of **Master of Science in Animal Science**.

DEDICATION

To my family,
you are my inspiration.

ABSTRACT

The objective of the work presented herein was to identify and fine map quantitative trait loci (QTL) for birth weight, pre-weaning average daily gain, and average daily gain on feed in a commercial line of *Bos taurus* using the identical-by-descent haplotype sharing method. A sample set of male calves of the Beefbooster, Inc., M1 line were typed using 71, approximately evenly spaced, microsatellite markers on bovine chromosomes (BTA) 2, 6, 14, 19, 21 and 23. Thirty-four haplotypes were found to have significant associations with the three growth traits. The haplotypes spanned fifteen chromosomal regions, two on BTA2 (9.1-22.5cM, 95- 100.3cM), three on BTA6 (8.2-50.1cM, 59.6-63.6cM, 83-86.2cM), three on BTA14 (16.5-25.5cM, 27-50.8cM, 52-67.7cM), three on BTA19 (4.8-15.9cM, 52-52.7cM, 65.1-65.7cM), two on BTA21 (9.9-20.4cM, 28.2-46.1cM) and two on BTA23 (23.9-36cM, 45.1-50.9cM). These QTL regions provide a reference for future positional candidate gene research and marker-assisted selection of growth traits.

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LIST OF SYMBOLS, NOMENCLATURE & ABBREVIATIONS

α -³³P – radiolabeled phosphorus

ABI – Applied BioSystems

ADGF – post-weaning average daily gain on feed

BAC – bacteria artificial chromosome

BoW – bone weight

bp – base pair

BTA – bovine chromosome

BWT – birth weight

BY – bone yield

CATS – comparative anchored tagged sequences

CCD – charge coupled device

cDNA – copy DNA

CLPG - callipyge

cM – centimorgan

COMPASS – comparative mapping by annotation and sequence similarity

dCTP – deoxycytosine triphosphate

DM – double-muscled

DNA – deoxyribonucleic acid

DP – dressing percentage

EBV – estimated breeding value

EST – expressed sequence tag

FISH – fluorescent *in situ* hybridization

FT – fat thickness

FTW – fat trim weight

FTY – fat trim yield

GDD – granddaughter design

G²DD – grand-granddaughter design

GDF-8 – growth differentiation factor 8

GH – growth hormone

GHR – growth hormone receptor

GHRH-R – growth hormone releasing hormone receptor

GLM – general linear model

HAS – human chromosome

HCW – hot carcass weight

H-FABP – heart fatty acid binding protein

hg – high growth

HGP – human genome project

IBD – identical-by-descent

IGF – insulin like growth factor

IMF – intra muscular fat

Kg – kilogram

LMA – longissimus muscle area

M – molar

MARC – Meat Animal Research Center

MAS – marker-assisted selection

mCi/mL – millicuries / milliliter

mh – muscular hypertrophy

MMU – mouse chromosome

MS – marbling score

ng/μl – nanograms per microliter

PCR – polymerase chain reaction

PWADG – pre-weaning average daily gain

QTL – quantitative trait locus/loci

RB – rib bone

RF – rib fat

RFLP – restriction fragment length polymorphism

RH – radiation hybrid

RM- rib muscle

RPW – retail product weight

RPY – retail product yield

SAS – Statistical Analysis Software

SCEUS – smallest conserved evolutionary unit segment

SSC – swine chromosome

Taq – *Thermus aquaticus*

WW – weaning weight

YAC – yeast artificial chromosome

YW – yearling weight

1. INTRODUCTION

Molecular genetics is an important area of scientific research that is continually growing and changing. Genetic information is crucial for the development of genome maps and the localization and isolation of genes of interest. Molecular technologies can be used to locate, identify, compare, or manipulate genes (Bourdon 2000). Now that physical and linkage maps have been established for a variety of species, localization of genes and examination of the biology and function of those genes, have become the focus of current research. Molecular technologies allow research at the level of individual genes, at the level of DNA, yet they continue to influence animal breeding and selection.

The bovine genome consists of 29 autosomes and the X and Y sex chromosomes. Over the past decade, a substantial amount of research has been done to identify, visualize, and analyze the bovine chromosomes and the genes located on them. First generation linkage maps were developed in 1994 for cattle (Barendse et al. 1994, Bishop et al. 1994). The male bovine genome, i.e. the entire set of male bovine genes, has been estimated to be 3567cM (cM = centimorgan, a measure of genetic distance that tells how far apart two genes are – two loci are said to be 1cM apart if recombination is observed between them in one percent of meioses (<http://ghr.nlm.nih.gov/glossary/centimorgan>)). The female bovine

genome, i.e. the entire set of female bovine genes, is an estimated 3765cM (Barendse et al. 1994). Second generation maps have also been developed (Barendse et al. 1997, Kappes et al. 1997). The consensus map of the bovine genetic map is estimated to span approximately 3500cM, contains over 1425 genetic markers, and has over 2850 mapped loci, with an average interval of 5.3cM between markers (Barendse et al. 1997, Kappes 1999).

Linkage maps show the chromosomal locations of specific genetic markers and genes of interest. A genetic map allows the mapping of specific genes such that if they lie between known markers their order and location are more easily determined. However, most maps were constructed using random markers and location was determined as a result of the analysis of those markers. The more markers present on a map the more likely it will be that a gene of interest will be closely linked to a known marker, and therefore more likely segregating with that marker. For markers to be informative they must be polymorphic in the population under study. A polymorphic marker must have subtle differences in the marker itself within a family, population, or breed, i.e. it must have at least two alternative forms or alleles. Polymorphic marker alleles are transmitted from generation to generation just as alleles of any particular gene.

Economics have produced a strong incentive in stimulating gene mapping of the cow, pig, sheep, and chicken (O'Brien et al. 1993). Most economic traits are quantitative. Quantitative traits are those traits considered to be controlled by a combination of many genes and the environment (Bourdon 2000). Important economic traits in the beef cattle industry include growth at all stages and carcass characteristics. These traits are important to both producers and to consumers, as well as to researchers. One of the goals of beef cattle molecular geneticists is to identify the genes, and the chromosomes on which they are located, which may affect growth at its many stages so that these genes can be identified and analyzed.

Quantitative trait loci (QTL) are defined as a single gene, or a small cluster of linked genes, that have an effect on a quantitative trait, which can be, but is not necessarily, a trait of economical value (Beever et al. 1990). For the development of genetic linkage and physical maps, the localization and identification of quantitative trait loci or genes, as well as the information obtained from comparative mapping, is important. As maps are constructed, genes can be isolated and analyzed to determine their overall biology and function.

Quantitative trait loci have recently been identified and mapped in experimental reference herds of beef cattle (Davis et al. 1998; Elo et al. 1999; Stone et al. 1999; Casas et al. 2000). These recent studies have focused on mapping QTLs affecting growth traits as well as other carcass

characteristics in beef cattle and other domestic livestock species (Charlier et al. 1995, Moody et al. 1995, Teres et al. 1996, Hetzel et al. 1997, Davis et al. 1998, Rodrigues et al. 1998, Taylor et al. 1998, Elo et al. 1999, Stone et al. 1999, Casas et al. 2000).

Recent studies have found significant evidence for QTL influencing various growth and carcass characteristics on many bovine chromosomes. A study by Davis et al. (1998) used a variety of genetic markers to detect regions in the bovine genome that accounted for variation in birth weight. Putative QTL were detected on five bovine chromosomes including BTA 5, 6, 14, 18 and 21. In a Brahman paternal half-sib family, evidence for QTL affecting growth and carcass characteristics were identified on BTA 1, 2, 5, 7, 11, 13, 14, 18 and 26 (Stone et al. 1999). In experimental reference herd families, of both Piedmontese and Belgian blue cattle, QTL were found to be segregating in regions of BTA 5, 6, 7, 13, 14, 17, 19, 22, 27, and 29 (Casas et al. 2000). Also, a QTL for live weight has been mapped to BTA 23 (Elo et al. 1999). Given that QTLs have been localized in experimental reference herds one can hypothesize that, QTL will also segregate in commercial beef cattle populations.

Beef cattle producers follow selection schemes based on what they would like to accomplish within their herds. Therefore, over time, certain traits, and thus genes and QTL, have been selected either for or against in commercial beef cattle populations. Commercial lines of cattle represent

semi-closed populations, typically derived from one or a limited number of founders. Therefore, common haplotypes (allele linkage phases determined by combining the allele of one locus, inherited from the sire, with the allele from the adjacent locus, also inherited from the sire), harboring QTL of interest, originating from these founders may carry on and segregate in the breeding line, especially when selection is applied. Common alleles that have segregated and carried on in a population from generation to generation make it possible to locate these QTL of interest.

The objective of this study was to identify and fine map quantitative trait loci for growth, in industry beef cattle herds, that have been previously identified in experimental reference herds. By recognizing and fine mapping QTL that affect growth one can detect positional candidate genes and gain a greater understanding of both their biology and their function. The results obtained in the identification and analysis of allelic variation in these genes would be useful in marker assisted selection (MAS).

Reported herein is the identification and fine mapping of QTLs within a commercial line of *Bos taurus* and their associations with birth weight (BWT), pre-weaning average daily gain (PWADG) and post-weaning average daily gain on feed (ADGF) using genetic markers of BTA 2, 6, 14, 19, 21 and 23. Previously, QTL for various growth and carcass characteristics were identified on these chromosomes (Casas et al. 2000; Davis et al. 1998; Elo et al. 1999; Stone et al. 1999).

2. LITERATURE REVIEW

2.1 Major Genes Affecting Cattle Growth

Most economic traits are polygenic, or quantitative. Quantitative traits are those traits thought to be controlled by many genes, by the interaction between genes, and by the interaction between genes and the environment. A gene is suspected to be a major gene if the difference between the mean value of the individuals with homozygous recessive alleles of the gene and that of individuals not carrying at least one recessive allele of the gene, is equal or superior to one phenotypic standard deviation of the trait of interest (de Vries et al. 1998). Major genes are those genes, which have a readily discernible effect on a trait; the effects of allelic variations of a single gene emerge as major changes in phenotype (Brewer & Sing 1983). Traits which were once assumed to be polygenic may actually be controlled by a combination of some major genes and many genes of smaller effect (Bourdon 2000). Major genes can be classified as either a single gene with a large effect, i.e. the trait of interest is under the influence of a major locus (Boehnke and Moll 1989), or as a small number of closely linked genes having a large effect (de Vries et al. 1998). The effects of such genes are also dependent on the interactions of the animal and its environment, as well as the interaction of the major gene with other genes. For example, Dam A, carrying alleles for high milk production, if not properly fed, will not reach her genetic potential for milk production;

Dam B, whose milk production is genetically inferior, but is well fed, could exhibit higher milk production than Dam A.

Major genes tend to segregate in intercrosses, thus the mating of phenotypically different individuals gives high heterozygosity and improves the information content of the population (Andersson-Eklund et al. 1998). The crossing of extreme phenotypes results in maximum heterozygosity at loci where the quantitative traits in question are affected, enabling the establishment of the mode and direction of allele segregation in the mapping population. If there is evidence for the segregation of a quantitative trait at a major locus then the locus could be mapped to a specific chromosome via linkage analysis.

The alleles of major genes can be detected by the analysis of phenotypic data from families in which the alleles segregate (segregation analysis). In addition to segregation analysis, molecular studies can be employed in order to identify the location of these major genes. For example, DNA markers can be used to detect the location of genes having substantial effects on quantitative traits. Major genes with effects on growth and development have been localized using gene markers. The traits associated with these major genes include double muscling in cattle (Charlier et al. 1995, Grobet et al. 1997), muscular hypertrophy in sheep (Cockett et al. 1994) and meat quality in pigs (Andersson et al. 1994). The utilization of both phenotypic data and

molecular genetic technology would allow researchers to fix, either for or against, major genes.

According to de Vries and associates (1998), the simple existence of major genes provides an opportunity for genetic improvement as it allows large steps to be made in the desired direction, it reduces variation among populations, and it allows for differentiation for specific markets. This is especially true with regards to growth and carcass characteristics of not only cattle but, any meat-type animal. For example, if it was determined that a particular gene was responsible for intramuscular fat deposition (fat within muscles, also referred to as IMF or marbling), then DNA tests could be employed such that marbling could be controlled. By determining which genes are major genes affecting cattle growth and carcass characteristics scientists are providing the opportunity for increasing the level of meat quality and decreasing variability in meat products.

One of the major genes affecting cattle growth is the *mh* (Muscular Hypertrophy) gene, also known as myostatin, or growth differentiation factor-8 (GDF-8). Myostatin is considered a major gene because of its extreme effect in the expression of growth and carcass traits (Casas et al. 2000). In addition, the *mh* gene is also considered a major gene because the difference between the postulated *+mh* and *mh/mh* animals is four standard deviations for some measures of muscularity (Charlier et al. 1995).

In a study, by Charlier and associates (1995), double muscled Belgian Blue sires were crossed with Dairy dams. The F1 dams were then mated to double muscled Belgian Blue sires in order to generate a segregating back-cross generation (i.e. offspring would be either $+/mh$ or mh/mh). This group was then able to identify a DNA fingerprint band that was cosegregating with the phenotype in offspring from a heterozygous sire, thus reinforcing the major gene hypothesis. They were thus able to localize the *mh* locus to bovine chromosome 2 (BTA2) via linkage analysis with a panel of microsatellite markers that covered the bovine genome; this evidence also confirms the existence of a major gene.

The *mh* phenotype is under the influence of an autosomal locus with both a wildtype and a recessive allele (Grobet et al. 1997). Cattle carrying two recessive alleles (homozygous recessive = mh/mh) exhibit a condition known as double muscling. This is common in Belgian Blue, Asturiana, and Piedmontese cattle breeds, among others (Grobet et al. 1997). Double muscled cattle tend to have reduced feed intake, improved feed conversion efficiency, and generally bring a substantially higher selling price (Cockett et al. 1994, Charlier et al. 1995, Grobet et al. 1997). However, the condition also tends to decrease milk yield, decrease female fertility, increase the incidence of dystocia, and increase susceptibility to respiratory illness.

Grobet and associates (1997) used a positional candidate approach to show that it is a mutation in the bovine myostatin gene, which causes the double

muscle phenotype. Myostatin (GDF-8) is a member of the transforming growth factor β superfamily of secreted growth and differentiation factors that is essential for proper regulation of skeletal muscle mass. The group developed comparative anchored tagged sequences (CATS), i.e. PCR primer pairs that amplify a sequence-tagged site from the homologous gene in a different species. These CATS were developed for a series of genes flanking particular RFLPs (restriction fragment length polymorphisms), which are located in the same chromosomal region as the *mh* locus. To test whether myostatin was associated with the double muscle phenotype the group designed primers based on available mouse and human sequences containing the myostatin gene. A frameshift deletion was detected in the gene. Individuals homozygous for the deletion exhibit the associated phenotype (Grobet et al. 1997). The identification of myostatin as the gene responsible for the phenotype has the potential to facilitate selection either for or against the double muscle trait; this could allow for the manipulation of muscle development. Grobet and colleagues (1997) suggest that if myostatin was identified as a key regulator of muscle development then this could permit the study of upstream and downstream factors, which could identify other genes potentially responsible for genetic variation in muscle development in livestock.

2.2 Quantitative trait loci

Quantitative trait loci (QTL) are defined as a single gene, or a small cluster of linked genes, that have an effect on a quantitative trait, which can be, but is not necessarily, a trait of economical value (Beever et al. 1990). It has been suggested that a few genes account for much of the genetic variation for quantitative traits and that trait variation is a result of the action of these alleles at many loci together with the action of environmental factors (Andersson et al. 1994). Much of the variability of quantitative traits is due to the segregation of a small number of QTL with large effects (Beever et al. 1990, Hetzel et al. 1997). Genotype can be deduced from phenotype because of this large gene effect; however, most quantitative traits are also under the influence of many loci, each having a smaller effect. Traits that are influenced by alleles at one or few loci are easier to localize using genetic mapping techniques (Andersson et al. 1996). The position of a QTL and its effect on genetic variation is established almost entirely on probabilities and statistical methods.

2.2.1 Genetic Markers

One type of genetic marker, or DNA marker, is genes. These are most valuable for comparative mapping as they are conserved across species, thus homologies can be established (Fries 1993, Andersson et al. 1996). These

genes have been given the term 'Type I anchor loci' and are useful in the construction of physical maps and comparative maps, and the localization of candidate genes (Fries 1993, O'Brien et al. 1993, Bishop et al. 1994). It is possible to select a group of anchor loci that act as reference markers for comparative genetic analysis. Type I loci are not as useful in the construction of genetic linkage maps as they are usually less polymorphic than Type II markers and may have little power in assessments of pedigree or population diversity (O'Brien et al. 1999).

The value of a genetic marker lies in its variation within a population. Markers need to be polymorphic and parents in a mapping population need to be heterozygous at the locus in order to be informative; highly polymorphic markers contain 6 or more alleles and show a minimum of 75% heterozygosity (Luty et al. 1990). A variety of markers that are not associated with known genes and exhibit high levels of variation are termed Type II markers. These are very important in the construction of detailed genetic maps (Fries 1993, Steffen et al. 1993, Bishop et al. 1994, Andersson et al. 1996). Included in these Type II markers are segments such as RFLPs (restriction fragment length polymorphism), microsatellites and RAPDs (random amplified polymorphic DNA). These markers are invaluable for increasing the genetic information content in selected pedigrees because there are thousands of them randomly dispersed throughout mammalian genomes and because each carries multiple alleles (O'Brien et al. 1993, O'Brien et al. 1999).

In DNA molecules there exist different sites (i.e. specific target nucleotide sequences) which are recognized by many restriction endonucleases. Restriction endonucleases cut at these sites producing small fragments of genomic DNA. Often these restriction sites are polymorphic (Griffiths et al. 1996). When a given restriction enzyme site is present in the DNA molecule of one chromosome but absent from the homologous chromosome, a shorter fragment is produced from the chromosome with the site and a longer fragment from the other chromosome (Watson et al. 1998). These

chromosomes can then be distinguished based on this restriction fragment length polymorphism (RFLP).

If an individual is heterozygous for such a chromosomal difference that region can be used as a marker in chromosome mapping (Griffiths et al. 1996). Restriction fragment length polymorphisms act as reference loci for mapping in relation to known genes or other RFLP loci. An individual that is heterozygous for an RFLP is said to be informative at that given locus. Given that the RFLP is inherited like a gene, it can be mapped and followed by tracing its inheritance pattern from generation to generation (Watson et al. 1998). Thus, the RFLP if it is located close to a known gene can act as a gene marker.

Restriction fragment length polymorphisms are not associated only with known genes; there is a distribution of RFLPs throughout the genome, thus they can be mapped by linkage analysis. Clues as to the location of a particular gene, in which the RFLP is occurring, come from comparing the inheritance of such markers of known location within a family. Interestingly, if two RFLPs reside, such that there is one on each side of a gene, the estimated position of that gene is more accurate. This is because the chance of a double crossover (i.e. two crossovers occurring in a chromosomal region) event is very small and thus both RFLPs and the gene will be kept together, and scientists know to look for the gene between the two flanking RFLPs (Watson et al. 1998).

Microsatellites are short repetitive segments of DNA, two to five nucleotides in length (dinucleotide/trinucleotide/tetranucleotide repeats) that repeat themselves 12 to 50 times. They are scattered throughout the genome. They can occur in the non-coding regions between genes or they can occur within genes (<http://ghr.nlm.nih.gov/glossary/microsatellite>). Parents in any given pedigree are likely to be heterozygous at such polymorphic loci, making these markers informative for linkage analysis and mapping (Fries 1993, Hawkins et al. 1995). Polymorphic microsatellites are useful in genotyping as PCR products are easily discernible by polyacrylamide gel electrophoresis making them easy to map to the genome (Fries 1993, Steffen et al. 1993). Microsatellite sequences are widely dispersed and highly repeated in eukaryotic genomes (Luty et al. 1990).

A set of microsatellites can be chosen to span either the entire genome or portions of the chromosomes in question. Such microsatellites are used as markers to map genes associated with certain traits. Microsatellites can be associated with known genes or can be anonymous (Fries 1993, Crawford et al. 1995). If a QTL is thought to be located on a particular chromosome then microsatellites mapped to that chromosome can be used to determine the location of the QTL. The more microsatellites, and the closer they are to one another, the greater the resolution of the area which they span. Once a large number of polymorphic microsatellites are mapped, the detection of QTLs affecting certain traits is possible. Panels of mapped microsatellites generated by the construction of genetic maps allows for the systemic searching of the

genome for QTLs segregating in a given population (Gao & Womack 1997, Hetzel et al. 1997, Kappes et al. 1997).

Early experiments screened the bovine genome for polymorphic microsatellites using computer software that would look for the presence of (CA)_n, (AC)_n, (GT)_n, and (TG)_n within the bovine genome database (Fries et al. 1990). Fries and associates (1990) predicted that because simple sequence repeats occur approximately every 10kb of DNA sequence, microsatellites could eventually be the markers of choice covering the bovine genome. The frequency with which microsatellites occur, the high degree of polymorphism associated with them, their distribution across the genome, and the fact that they can be easily isolated and characterized potentially make them very useful as markers in gene mapping studies (Moore et al. 1991).

Another type of genetic marker being utilized in genome studies is the single nucleotide polymorphism (SNP). As the name suggests, these are the result of a mutation from the original specific nucleotide present in a particular location in an individual to a different nucleotide at that same site and can be transmitted from parent to offspring, just like any other gene (www.beefimprovement.org/guidelines/Chap4.pdf). For a variation to be considered a SNP, it must occur at a frequency of 1% in the mapping population. Many thousands of SNPs exist, such that they occur, approximately every 100 to 300 bases in the human genome. Over 2.8million SNPs have been identified in the human genome, a similar number are expected to exist in the bovine

genome. Due to their wide distribution in the genome, it is likely that any gene of economic importance is located closely adjacent to several of them that can be used to mark its presence. Some SNPs are located within the coding sequence of a gene and therefore can affect the structure and function of a protein. This variation could be responsible for differences among individuals in phenotypic merit for economically important traits. Other SNPs occurring in non-coding regions and may regulate gene expression. Single nucleotide polymorphisms are advantageous genetic markers because they are widely distributed throughout the genome, occur frequently throughout the genome, they are less likely to undergo spontaneous mutation and are inherited with greater stability than microsatellite markers (because they have a low rate of mutation per generation). While microsatellite marker maps of domestic animals can provide average intermarker distances of 1-2cM a SNP map could provide a thousand times greater marker density even up to an average intermarker distance of 300 base pairs (Haley 1999). Therefore, a genome map based on SNPs would have a higher resolution than a map of microsatellite markers, thus facilitating fine mapping of QTLs.

2.2.2 Molecular techniques in gene mapping

In order to map the human, mouse, and livestock genomes a number of molecular techniques have been employed to aid in the process. Traditional molecular techniques included the use of somatic cell hybrid panels, in situ

hybridization, and pedigree analysis; newer approaches include the use of radiation hybrid panels, DNA segment cloning, yeast / bacteria artificial chromosomes (YAC / BAC) contig alignment, positional cloning, and interspecies chromosome painting (Zoo-FISH) among others (O'Brien et al. 1999). One molecular technique not specific to gene mapping but used in conjunction with those techniques is the polymerase chain reaction (PCR). Standard PCR is the amplification of genomic or plasmid DNA using a standard *Thermus aquaticus* (*Taq*) DNA polymerase.

The polymerase chain reaction (PCR) technique was developed by Kary Mullis in the mid 1980's. It has since revolutionized molecular genetics (Watson et al. 1998). Using PCR, DNA can be amplified to large amounts (Mullis et al 1986). In the PCR technique, single-stranded DNA acts as a template for the synthesis of a complementary new strand. The DNA for a PCR can come from a number of sources including genomic DNA, cDNA, plasmid vectors, and bacteriophage vectors, among others. In order to use PCR, one must design DNA sequences, referred to as primers, which flank (lie on either side of) both ends of a given region of interest in DNA (may be a gene or any sequence). When the primers and DNA anneal, two 3' ends will be facing each other. An original double-stranded DNA sequence is heated to separate the strands; a forward primer and a reverse primer anneal to their appropriate strand and new strands of DNA are synthesized via the action of *Taq* polymerase. The reaction mixture goes through a series of repeated cycles of primer annealing, DNA synthesis, and denaturation. After n cycles,

if starting with A DNA molecules, the reaction theoretically contains $A \cdot 2^n$ double-stranded DNA molecules (Watson et al. 1998). The primary goal of PCR is to amplify a small amount of DNA into a much larger amount, in order to produce enough DNA to be used in experimental procedures.

2.2.2.1 Somatic cell hybrid panel analysis

Cells that are growing in tissue culture can be made to fuse with cells from another species. By mixing suspensions of cattle cells and mouse cells, for example, and an inactivated virus, fusion of the cells can occur producing a uninucleate cell line composed of both species chromosome sets (Griffiths et al. 1996). Because the chromosomes of the two species are recognizably different, often in number and size, in hybrid cells, the two chromosome sets can be distinguished. When bovine-rodent fusions are made and the hybrid cells are grown in a culture, there is a progressive loss of the bovine chromosomes until some stable but random state is reached in which only one or a few bovine chromosomes are left (Watson et al. 1998). Single hybrid cells can be isolated and grown to make lines of rodent cells containing a small amount of bovine DNA. Hybrid cell lines containing the bovine chromosomes carry either a different complement of mouse chromosomes or different fragments of the same chromosome, the fragments overlap such that the bovine chromosome can be defined and mapped.

Moody and associates (1995) established a panel of bovine-rodent hybrid cell lines to assign selected genes to particular bovine chromosomes. The

presence or absence of a bovine-specific PCR product was tested. Segregation pattern profiles were compared with previously assigned markers. The somatic cell hybrids were used to confirm the location of growth hormone receptor (GHR) on bovine chromosome 20 (BTA 20).

In a technique referred to as radiation hybrid (RH) mapping, x-irradiation is used to break up the chromosomes in bovine cells. These fragments can be fused with non-irradiated rodent cells and a selectable marker is then used to identify hybrid clones containing the bovine DNA fragments from the area with the marker; thus it is possible to determine which markers are inherited together and therefore linked (O'Brien et al. 1999). Neither pedigree information nor marker polymorphism is required, therefore facilitating the mapping of any unique markers or genes that can be differentiated from the host genome, regardless of allelic variation (Yang et al. 1998).

Womack et al. (1997) created a bovine 5000-rad whole genome radiation hybrid (WG-RH) panel. By analyzing a large number of these cell lines marker, or gene, order can be determined for all chromosomes. The primary objective was to integrate linkage maps of microsatellites with evolutionarily conserved genes into one ordered map to permit comparative genome mapping at a higher level of resolution and eventually facilitate the cloning of QTLs. Linkage maps tend to be independently derived and are not highly integrated through the use of common markers. The range of resolution of a

radiation hybrid map is dependent upon the radiation dosage used to make the RH panel; therefore, RH maps have higher resolution than linkage maps.

Using the panel created by Womack and colleagues (1997), Band and associates (1998) developed an RH map of BTA 23 that also complemented, and integrated markers from existing maps. High resolution of the map allowed the reliable ordering of markers including 13 microsatellites and 13 Type I genes. Yang and associates (1998) also utilized the panel to construct a RH map of BTA 19, on which they placed 15 microsatellite markers, 15 coding genes and 2 ESTs. Ozawa and colleagues (2000) used a whole genome cattle-hamster RH cell panel to construct a RH map of 54 markers on BTA5. The markers included 34 microsatellites, 6 ESTs, and 14 genes. Of the 14 genes, 10 were new assignments that had been selected based on genes mapping to HSA12 and HSA22 that were predicted to map to BTA5, on the basis of comparative mapping. The placing of these markers on a map of BTA5 greatly improved the resolution of the BTA5 gene map and revealed previously unknown details of the cattle-human and mouse-human comparative maps (Ozawa et al. 2000).

More recently, Band and associates (2000) have placed a total of 768 genes and 319 microsatellites on the RH map. Of these, 638 had human orthologs, thus permitting the construction of a comparative map. Using a technique referred to as comparative mapping by annotation and sequence similarity (COMPASS) the group found that the identification of the location of cattle

chromosome location from random sequence data was 95% accurate. Radiation hybrid mapping appears to be an ideal tool for placing conserved genes on an ordered map and at the same time integrating existing linkage maps of microsatellites.

2.2.2.2 Yeast / Bacterial artificial chromosomes

Yeast / bacterial artificial chromosomes (YAC / BAC) are yeast / bacteria vectors that contain a centromere and two telomeres; the centromeres mediate stable replication and partitioning of chromosomes at mitosis and meiosis, the telomeres (ends of the chromosome) are also required for replication and stability and are responsible for permitting the YAC / BAC to behave as a linear chromosome (Watson et al. 1998). These vectors can carry many kilobases of DNA and then replicate, such that many clones are developed. Because yeast and bacteria centromeres and telomeres have been cloned and characterized, they can be used to assemble artificial chromosomes that carry large segments of DNA from a donor genome and make new clones containing the donor genes. These new clones can then be screened on a large scale by pooling many clones and using the PCR technique to identify specific genes; or, clones can be plated and tested by hybridization (Gallagher et al. 1998, Watson et al. 1998).

2.2.2.3 Positional cloning

Positional cloning can be very useful if a quantitative trait locus (QTL) has been established but there exists some uncertainty of which genes reside in that location. The cloning of a gene that has no information regarding its protein product begins with determining the chromosomal location of the gene (Watson et al. 1998). Positional cloning uses tightly linked markers as entry points to 'walk' along the chromosome towards the gene of interest (Andersson et al. 1996). Information regarding the gene's location is used to clone the DNA sequences in that area and these sequences are then used as probes to find the gene itself. Once the gene is isolated, it can be sequenced and eventually analyzed to determine the characteristics of the protein for which it codes (Watson et al. 1998).

2.2.2.4 In situ hybridization

In situ hybridization is used to locate cloned genes and markers. If individual chromosomes of a genomic set are recognizable by their banding pattern, size, or other cytological feature, then genes can be assigned to the chromosome it hybridizes to (Womack 1989, Griffiths et al. 1996); this also reveals a rough estimate of the chromosomal position of the gene. Sequences of DNA can be localized to sub-chromosomal regions by hybridizing radioactively labeled probes directly to chromosome spreads. Chromosomes are treated such that cell division is blocked at metaphase. This ensures that homologous chromosomes are paired and visible. Metaphase spreads are obtained from

bovine fibroblast cell lines and stained with Giemsa to identify the chromosomes before hybridization occurs (Hediger et al. 1990). Chromosomes treated with Giemsa show a pattern of light and dark bands for each chromosome; chromosomes can be identified individually in this way (Watson et al. 1998). The location of the radioactive probe is revealed by the distribution of colored grains in a photographic emulsion that is layered over the spread (Hediger et al. 1990, Watson et al. 1998). This staining of the chromosomes reveals their banding pattern and can show where certain genes or DNA segments are located.

In fluorescent in situ hybridization (FISH), a probe is coupled to a fluorescent molecule. A partially denatured chromosome preparation is bathed in the probe. The probe binds to the chromosome and the location of the selected fragment, or gene, is revealed by a bright fluorescent spot (Griffiths et al. 1996). Fluorescent labeling also requires the use of one or more mapped probes as landmarks on chromosomes, as these are more difficult to visualize. The location and order of several probes can be determined simultaneously if they are labeled with different fluorochromes (Watson et al. 1998). This is very useful in mapping the position of markers and genes along a chromosome.

Cross-species comparisons can also be made using FISH. The painting of a chromosome in one species allows identification of homology with chromosomes of other species; this is often referred to as Zoo-FISH or

interspecies chromosome painting (Andersson et al. 1996, O'Brien et al. 1999). In this variation of the in situ hybridization technique, DNA from fluorescently-labeled flow sorted individual chromosomes of one species is hybridized in situ to metaphase spreads of a compared species (O'Brien et al. 1999). Using Zoo-FISH it is possible to detect small regions of homology and then construct comparative maps using this information.

2.2.3 Genetic maps in beef cattle

Gene maps have been constructed as both a resource for locating the genetic determinants of heritable characteristics, behaviors, and phenotypes, and as a template for resolving and interpreting the patterns of evolving genome organization in their ancestry (O'Brien et al. 1999). Genome maps include both linkage maps and physical maps. The ideal goal of mapping projects is to create a dense ordered map of each chromosome by integrating the various types of markers.

A linkage map is essentially a road map of all the chromosomes of a species, and contains the linear order and genetic spacing of genes or genetic markers for each chromosome. Linkage maps always contain polymorphic markers because of their associated allelic variation (Andersson et al. 1996). A physical map indicates the location of genes or genetic markers on each chromosome and may contain cytogenetic (pictorial) information on the chromosome and/or the chromosome banding pattern. The lowest resolution physical map is the banding pattern of each chromosome, the ultimate

physical map would be the complete nucleotide sequence for each chromosome.

The entire bovine genome can be mapped both genetically and physically, and scientists have also developed both male and female maps; which is possible due to differences in the X and Y chromosome's, as well as differences in recombination rates for male and female cattle. In addition, maps of individual chromosomes have also been developed. Currently, genetic maps of both individual chromosomes, and genomes of livestock are available, but there exist gaps in both that are being researched and filled in. Extensive genetic and physical maps of each chromosome in the bovine genome are proving to be very useful in the mapping of specific genes. Various methods have been employed for constructing such linkage and physical maps.

Linkage maps are constructed based on the principle that there is the coinheritance of certain markers and genes and this can be followed within families (Fries 1993, Watson et al. 1998). If the alleles at two loci tend to cosegregate in progeny of animals heterozygous at those loci, then the two loci are likely linked. Linkage is determined by analyzing the pattern of inheritance of alleles of genes or of markers in suitable families. Linkage maps provide information relative to the distance between genes and the frequency of crossover events. Recombination refers to any process in a diploid cell or partially diploid cell that generates new gene or chromosomal combinations not found in that cell or its progenitors (Griffiths et al. 1996).

Recombination is more likely between markers that are further apart, the more closely linked the less likely recombination will occur. In order to be efficient a marker map should consist of highly polymorphic loci no more than 40cM apart (Fries 1993).

Markers are placed on a map relative to one another and order is determined by recombination between them (Watson et al. 1998). Markers are used relative to one another in order to indicate the location of genes on the map encompassing the chromosomes of a given species. Linkage maps can then be used to refine the location of a particular locus and identify the genes influencing a particular trait. This is especially useful for mapping areas that contain genes or QTLs affecting production traits (Barendse et al. 1994, Stone et al. 1999). The primary reason for developing a bovine linkage map is to provide markers that are genetically linked to economically important traits so that linkage relationships can be established and appropriate genes mapped (Womack 1989).

Microsatellites are highly polymorphic and therefore provide the information required to construct good linkage maps. Microsatellites have been widely developed and used to map syntenic groups, chromosomes, genes, and QTLs. Genetic linkage maps are based almost entirely on microsatellite markers. Microsatellites may span anywhere from a specific region on a chromosome to the entire genome. For mapping individual chromosomes, the number of polymorphic microsatellite markers ranges from 5 to 36, but is typically

between 6 and 15 (Vaiman et al. 1994, Yeh et al. 1996, Gao & Womack 1997, Sonstegard et al. 1997, Wang et al. 1998, Connor et al. 1999, Elo et al. 1999, Kuhn et al. 1999). Current experiments utilizing whole genome scans generally use between 150 and 250 microsatellite markers in order to generate a map with <20cM intervals between the markers; this is so that genes and QTLs can be mapped as close as possible to their chromosomal position (Charlier et al. 1995, Hetzel et al. 1997, Stone et al. 1999, Casas et al. 2000).

When the search for the location of QTLs first began the number of polymorphic microsatellites was small, and thus map marker density was low. The need for more markers to increase map density and subsequently locate desired QTLs was, and still is, great. Dense marker maps, and advanced statistical methods make QTL searching feasible. A genetic linkage map covering the entire genome with sufficient informative markers is required for a thorough search of loci. Such a map proves useful in defining the location and characterization of the loci and the genes affecting a particular trait (Moore et al. 1994, Sonstegard et al. 1997, Arranz et al. 1998, Kappes 1999, Stone et al. 1999). Kappes (1999) suggests that this is best accomplished by determining the segregation patterns of DNA segments and phenotypes over two or more generations and then utilizing these maps for identifying regions that affect economic traits.

Physical maps are constructed by a variety of methods including the analysis of chromosome abnormalities, the analysis of somatic cell hybrid panels, and

the utilization of other methods such as in situ hybridization that allow for the direct assignment of genes to chromosomes (Fries 1993, Echard et al. 1994, Vaiman et al. 1994, Andersson et al. 1996). Utilization of the PCR technique, to screen somatic cell hybrid panels, results in the identification of groups of genes lost or retained together, i.e. they are syntenic (Fries 1993). Early experiments mapped known genes to these syntenic groups, which were then mapped to chromosomes on which anchor loci had been located. Over the past decade all the syntenic chromosomes have been defined, primarily through hybridization methods (Bishop et al. 1994, Eggen & Fries 1995, Ferretti et al. 1997, Larsen et al. 1999).

In domestic animals one of the goals of genome mapping is to identify and characterize the genes controlling variation in traits of economic interest (Elo et al. 1999). Generation after generation of livestock has continually undergone selection with the goal of improving such traits. Most economic traits are under the influence of many genes and are influenced by the environment, thus they are quantitative. The common economic traits measured in the beef cattle industry include growth at various stages and carcass characteristics. Carcass composition (including fat deposition, lean tissue yield, etc) and meat quality (includes muscle tenderness, palatability, etc) are key determinants of carcass value and consumer satisfaction. The key limiting factor is that the majority of these traits cannot be measured in live animals. Therefore, there is a great interest in localizing the QTL affecting

variation in these traits in order for marker-assisted selection to take place (Taylor et al. 1998).

The genetic linkage maps of cattle are reaching a mature phase. The initial goal in creating a bovine genetic linkage map was to construct a map with average spacing of 20cM, to search for the location of genes affecting economically important traits and provide information on genomic evolution (Barendse & Fries). The first two genetic maps of cattle (Barendse et al. 1994, Bishop et al. 1994) genotyped at random 202 and 213 loci respectively. Later versions of these two maps, as well as additional independent mapping efforts have resulted in more than 1600 loci being mapped in the bovine genome (Georges et al. 1995, Ma et al. 1996, Barendse et al. 1997, Kappes et al. 1997). The average marker density for cattle maps is 2.5-3.5cM. Nevertheless, there are regions with low densities of markers, thus there exist gaps that still need to be filled in.

2.2.4 QTLs for growth and carcass traits in beef cattle

Polymorphic microsatellites have also been used to map QTL related to growth and carcass characteristics in cattle. Such markers can be used to detect the genes responsible for genetic variation in these traits in various populations. Hetzel and colleagues (1997) wanted to detect QTL for carcass and meat quality traits in a herd of tropical beef cattle. This group found QTL

for 18 out of 54 carcass and meat quality traits, using available microsatellite markers and three large paternal half-sib families. The group concluded that the gene markers used were useful genetic predictors for traits.

Selection indices that included QTLs with accurately estimated effects on such traits could provide a means of genetic evaluation early in life (Stone et al. 1999) and this would be useful in permitting a more direct approach in integrating production practices with market demands. This would be especially useful in populations segregating QTLs with major effects, in that individuals could be sorted early in the production cycle to match genotypes with production targets, and thus finishing programs. By mapping the genes affecting these traits using molecular genetics techniques, versus the traditional use of phenotypic and pedigree data, it would be possible to select genetically superior individuals quickly and efficiently.

It is useful to identify a population in which the QTL and markers of interest are segregating because only these provide necessary linkage information (Boehnke & Moll 1989). In dairy cattle populations where the males do not express traits of interest, experiments utilize either a granddaughter design (GDD) or a grand-granddaughter design (G^2DD) to confirm the segregation of QTLs in specific sire families (Weller et al. 1990, Arranz et al. 1998, Ashwell et al. 1998, Zhang et al. 1998). Early work with QTLs was done in Dairy cattle, where milk production traits are expressed only in females. In the GDD method, measurements are taken utilizing paternal half-sib information

from different families. This method is sufficient in determining moderate QTL as long as multiple grandsire families are included with many sons. The principle of the G^2DD method is that, if a segregating QTL causes a phenotypic contrast of δ between sons inheriting alternate QTL alleles from their heterozygous sire, the same phenotypic contrast, δ , is expected among grandsons having inherited alternate alleles from their grandsire. This allows for tracing the segregation of the founder sire's chromosomes via its ungenotyped daughters to its grandsons using available genetic markers (Arranz et al. 1998). Arranz and associates (1998) used grandsons in the G^2DD analysis whose grandsires showed evidence for QTL segregation in the GDD method. Once a chromosome segment is determined to harbor a QTL, additional chromosomal regions need to be examined, and further screening of the entire progeny of many families needs to be pursued to identify QTL in other chromosomal regions (Casas et al. 2000).

Much work has been done to map QTLs in cattle with respect to growth and carcass characteristics. Early work by Beever and colleagues (1990) showed an association of 205 day and 365 day weights, pre-weaning average daily gain (ADG), and subcutaneous fat thickness to the RBC-B system on BTA12. It was determined that half-sibs inheriting the paternal RBC-B system had heavier weaning and yearling weights, higher ADGs, less back fat, and more desirable yield grades. Therefore, it was hypothesized that a QTL affecting these traits was linked to or within the RBC system located on BTA12. Rocha and associates (1992) found a QTL associated with dam genotype affecting

calf weaning weight, and dam genotype affecting calf birth weight on BTA15, and BTA19, respectively. These QTL effects were localized relative to the position of the parathyroid hormone gene and the growth hormone gene, respectively.

Live weight is a major determinant of carcass value and is highly heritable, thus it is responsive to selection. Weights at all ages are correlated therefore the selection for increased mature weight often results in increased birth weight, which can reduce productivity due calving difficulties. Thus, it would be useful to find QTLs that influence the developmental regulation of growth such that mature weight could be influenced without influencing birth weight (Taylor et al. 1998). Elo and colleagues (1999) have mapped a QTL for liveweight to BTA23. It has been shown that the major histocompatibility complex also resides on this chromosome. In many species, including mice, chickens, and swine, the MHC haplotype is associated with growth traits (Beever et al. 1990).

Stone and associates (1999) conducted a primary genomic screen for QTL affecting carcass and growth traits via genotyping 238 microsatellites on progeny from a *Bos indicus* X *Bos taurus* sire mated to *Bos taurus* cows. The data was analyzed to determine if QTL for birth weight (BW), weaning weight (WW), yearling weight (YW), hot carcass weight (HCW), dressing percentage (DP), fat thickness (FT), marbling score (MS), longissimus muscle area (LMA), rib bone (RB), rib fat (RF), rib muscle (RM), retail product yield

(RPY), fat trim yield (FTY), bone yield (BY), retail product weight (RPW), fat trim weight (FTW), and bone weight (BoW) could be identified and mapped.

The aforementioned study utilized Brahman and Hereford cattle. It was found that there were QTLs on BTA5 for increasing RB, BY, BoW, and BW if the Brahman allele segregated from the sire. Also located on BTA5 were QTLs for decreasing DP, RF, MS, and FTY for those calves with segregating Brahman sire alleles at these loci. Four QTLs affecting the proportion of fat and muscle in wholesale rib cuts were mapped to BTA2 in individuals with segregating Brahman alleles. Another four QTLs reside on BTA13, two QTLs on BTA18 and BTA26 each, and one QTL on each of BTA11, and BTA14 - and these too influence carcass characteristics. The QTLs influencing BY and DP on BTA5, and DP on BTA13 share map positions with the genes involved in the GH-IGF endocrine axis (GH and IGF could act as candidate genes for mapping these QTL). The region of BTA5 considered here is syntenic to the region of MMU10 containing the *hg* gene. This group also found a QTL on BTA1, believed to be responsible for birth weight, when the Brahman allele segregates. According to Stone and associates (1999) a secondary screening involving more individuals and more genetic markers is required to verify the QTLs identified and to determine the effect and frequency of alleles influencing these.

Casas and associates (2000) work has identified the further mapping of many QTLs to various chromosomes. This group utilized two families – one with a Belgian Blue X MARC III sire ($\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Red Poll, $\frac{1}{4}$ Pinzgauer), and one with a Piedmontese X Angus sire. Both the Belgian Blue and Piedmontese breeds have two inactive myostatin alleles resulting in a condition known as double muscling. Due to the crossbred nature of the sires, it is assumed that they are heterozygous at the myostatin gene. Although myostatin is greatly associated with the variation on muscle mass and fat deposition in double muscled cattle populations there are likely additional loci responsible for much of the variation in these characteristics. It is also assumed that half of the progeny in each family would receive an active myostatin gene copy and half would receive an inactive copy of the myostatin gene. Offspring were then evaluated for growth and carcass characteristics.

For offspring with Belgian Blue inheritance QTLs for carcass traits were localized to BTA5, BTA6, BTA7, BTA19, BTA27, and BTA29. In addition, QTLs for growth were identified on BTA6 and BTA22. Piedmontese inheritance resulted in offspring with QTL for carcass traits on BTA5, BTA7, BTA13, BTA14, BTA17, BTA22, and BTA29. This group also suggested that the QTLs for carcass traits on BTA5 are closely linked to the IGF-1 gene. One QTL for birth weight was localized on BTA19 in offspring with segregating Piedmontese alleles. Offspring inheriting an active myostatin gene from Piedmontese sires on BTA14 were leaner than those inheriting the Angus allele.

Initial mapping of QTLs required crosses between individuals of known accurate phenotypes, large pedigrees, and many informative markers. Without the utilization of individuals with accurate phenotypes, it is difficult to determine which alleles are segregating. The use of larger pedigrees ensures a higher power of detecting segregating QTLs (Hetzel et al. 1997, Wang et al. 1998). With small sample sizes only QTLs with large effects will be detected, by increasing the sample size QTL with moderate to low effects can be detected. The more polymorphic microsatellite markers there are scanning the bovine genome, combined with a larger sample size with more informative meioses, the more accurate the determined position and effect of the QTL.

Currently sample sizes and resources are not large enough. More families, and more individuals must be genotyped, and more markers that are informative need to be identified, in regions where QTLs are thought to exist. Once these things are accomplished then saturating a region with markers, in order to fine map and clone a QTL can be undertaken (Zhang et al. 1998). According to Riquet and colleagues (1999), because fine mapping techniques are rather limited, the cloning of QTLs has been delayed. The primary goal now is to be able to clone QTLs such that they can be mapped, and thus utilized, in both experimental and commercial outbred cattle populations (Riquet et al. 1999).

2.2.5 Comparative mapping

Andersson and colleagues (1996) suggested that the ideal situation would be to integrate the genetic linkage map and the physical map to show homologous segments and then use linkage analysis to identify genes that were responsible for homologous phenotypic traits between species. If a certain production trait could be mapped in the mouse genome, then by comparing that with the bovine genome it would be possible to genetically and physically map the location at which the gene would theoretically reside. This 'comparative mapping' has now become common practice.

Many studies have employed the use of either a bovine/human hybrid cell panel, or a bovine/rodent hybrid cell panel to develop synteny maps and localize genes. Hybrid clones from the somatic cell panel are tested for their ability to amplify predicted fragments by PCR with primers from bovine sequences (Dietz et al. 1992). The presence of an amplified fragment represents the presence of bovine template, thus determining the syntenic assignment for each genetic marker. It is also possible to use a short piece of the 3' or 5' end of a gene from a cDNA library, commonly referred to as expressed sequence tags (EST's), which can be mapped quickly and are useful due to sequence similarity of homologous genes in different species (Kappes 1999).

Syntenic comparisons among distantly related species, suggests that large chromosomal regions are conserved within mammals and over many

generations (O'Brien & Marshall Graves 1990, Dietz et al. 1992). These are typically referred to as regions of synteny. Mammals have a highly conserved genome size. Originally, mammalian genomes were expected to share somewhere between 70,000 and 100,000 genes, this has since been reduced to 30,000 genes (O'Brien & Marshall Graves 1990, Andersson et al. 1996). Conserved synteny occurs when the same set of genes grouped together on a chromosome in one species is found grouped together on a chromosome in another species. These conserved groups of genes are useful guides to the locations of similar genes in other species. Moore and associates (1991) found, via searching DNA sequence databases, that the locations of microsatellites are often conserved among closely related mammalian species. This makes them useful in comparative mapping strategies (Moore et al. 1991, Kemp et al. 1993).

There are two distinct goals in comparative mapping strategies: 1) to provide a resource for genetic analysis and 2) to help dissect the evolution of genome organization by comparing linkage relationships of homologous genes (O'Brien & Marshall Graves 1990). The mouse and human genomes are likely to continue to be the primary models for gene mapping in other species, even though they are not entirely representative (O'Brien et al. 1993). The strength of the comparative genetic approach is derived from connecting the dense gene-rich maps of human and mouse with agricultural species maps even though livestock maps are less dense (O'Brien et al. 1999). Comparative mapping is important in elucidating the rules that underlie genome

architecture and the evolution of karyotypes. It also has a strategic function in positional cloning approaches in domestic species (Solinas-Toldo et al. 1995). It is possible that desirable alleles from one livestock species could be used to improve production traits in other species. Therefore, the identification of QTL for growth and carcass characteristics in one species will be useful for the localization of such QTL in other species (Andersson et al. 1996). The identification of genes affecting the regulation of growth in mice has relevance in livestock improvement.

O'Brien and associates (1993) utilized 321 anchored loci based on cloned mouse and human functional genes, spaced at an average of 5cm and 10cM throughout their respective genomes, to build a map that would provide the basis for a unified approach to comparative analysis of mammalian genomes. This group defined clusters of homologous genes linked together in two species as a conserved evolutionary unit segment (CEUS). When such a segment went uninterrupted in gene maps of other species it was recognized as the smallest conserved evolutionary unit segment (SCEUS). Flanking markers and internal genes from the SCEUS region were added to preserve the 5-10cM spacing in the constructed map. In addition, a group of candidate markers, between which recombination had not been detected was selected, and the marker most informative for linkage to other loci was chosen to represent that genomic region (O'Brien et al. 1993).

Human loci can be aligned with corresponding locations in a particular livestock species, and mouse to develop a very informative 3-way map. Genes are selected based on an associated phenotype; therefore, mouse/human genes can be used to select cattle/pig/sheep genes (Kappes 1999). Once the chromosomal region to which a particular gene resides has been mapped, and it is known which chromosomes between species are syntenic, the information can be used to map that gene between species.

Comparative mapping is important for integrating information from a diverse range of species, such that if a gene is found in one species, relevant information can be easily and efficiently obtained, in another species. Dense genetic maps based on coding genes and microsatellites have been complemented by precise gene homolog alignment with moderate resolution maps of livestock, companion animals, and other mammalian species (O'Brien et al. 1999). The use of radiation hybrid mapping has allowed for the ordering of conserved genes on comparative maps at a higher resolution than what was traditionally possible. Radiation hybrid mapping appears to be an ideal method for placing conserved genes on an ordered map and at the same time integrating existing linkage maps of microsatellites (Yang et al. 1998).

The most recent ordered comparative map of the cattle and human genomes has been created by Band and associates (2000). As mentioned previously, it is a whole-genome radiation hybrid map containing 638 orthologous markers,

out of a total of 768 cattle genes and 319 cattle microsatellites. The comparative map generated will be a cornerstone for elucidating mammalian chromosome phylogeny and the identification of genes of agricultural importance (Band et al. 2000).

2.2.5.1 Bovine genome map versus human genome map

The focus in mapping the human genome was on the normal functioning of genes, and the abnormal functioning of genes causing genetic disease (Andersson et al. 1996). These initial studies proved useful in the mapping of cattle chromosomes and genes as well, even though the focus can be very different. In cattle, research tends to focus on economic traits such as growth, fatness, and reproduction.

Early comparative mapping studies focused on developing a synteny map. Markers were selected based on physiological significance in cattle, potential for filling in the gaps in the comparative map, and the availability of published sequences for synthesizing PCR primers. Many regions of conserved synteny have been mapped between the bovine and the human genome. The use of fluorescence in situ hybridization (FISH) paints regions of homology, to establish comparative genome maps and delineate conserved segments (Solinas-Toldo et al. 1995).

By comparing the order of loci on cattle chromosomes with the corresponding order on human chromosomes, there is evidence of rearrangement within

conserved syntenic groups (Barendse et al. 1993). The primary use of the comparative bovine-human map will be to identify candidate genes in the regions of the bovine chromosomes that contain trait loci of economic importance (Solinas-Toldo et al. 1995).

Charlier and associates (1995) determined that the majority of bovine chromosome 2 (BTA2) corresponds to human chromosome 2. Interestingly, this group found a microsatellite (TGLA44) closely linked to the *mh* locus. Therefore it is likely that the human homolog of the *mh* locus is located on HSA 2. Moody and associates (1995) discovered that the growth hormone receptor (GHR) gene is located on HSA5, which is partially homologous to BTA20. Fibroblast growth factor receptor 3 (FGFR3), in humans maps to HSA4, in cattle it maps to BTA6 (Teres et al. 1996). Insulin-like growth factor 1 receptor (IGF-1R) on BTA21 is homologous to HSA15 (Moody et al. 1996). Another important discovery was the assignment of the bovine obese gene to a linkage group on BTA4, which is syntenic to HSA7 (Stone et al. 1996). In addition, growth hormone releasing hormone receptor (GHRH-R) has also been mapped to this group. Additional research shows similar results as those identified here, as well as other comparisons between various chromosomes. The knowledge of human genetics leads genomics in such a way that human gene regulation and orientation inform us as to animal gene action (O'Brien et al. 1999).

2.2.5.2 Bovine genome map versus murine genome map

The murine (mouse) genome has been studied at length, and next to the human genome, is the most widely understood and extensively mapped. Of particular interest are the genes, in the murine genome, that control growth and carcass characteristics and how these compare to the bovine genome.

Information from the mouse genome has been used to identify genes in cattle. The deposition of fat in livestock is an important issue, not only with respect to efficient production but also with respect to consumer concerns and demands. The obese gene in mice was found to reside on mouse chromosome 6 (MMU6). The bovine homolog of the obese gene was mapped to a linkage group on BTA4 (Stone et al. 1996).

In addition to fat deposition, the accretion of muscle in livestock is also important. The *mh* (muscular hypertrophy), or myostatin, gene is responsible for double muscling in cattle. McPherron et al. (1997) explain that the homozygous deletion of myostatin in mice causes extreme cellular hyperplasia, which is similar to double muscling. Mutations in the myostatin gene cause the double muscled phenotype to be displayed because the myostatin protein is inactivated (Grobet et al. 1997). Grobet and associates (1997) used a positional candidate gene approach to identify that a mutation in bovine myostatin is responsible for the double muscling phenotype. The mouse myostatin gene sequence, located on MMU1, was used to identify a

clone that contained cattle myostatin and the cattle gene was sequenced and mapped to the centromeric end of BTA2.

Casas and colleagues (2000) performed research concerning the presence of quantitative trait loci (QTLs) for carcass traits and birthweight. They found that QTLs for fat depth, retail product yield and yield grade were located near the IGF-1 gene on BTA5, and that a QTL for growth in mice was also located near its IGF-1 gene, which is located on MMU10. Also located on MMU10, near the IGF-1 gene, is a major gene referred to as the “high growth” (*hg*) gene. This gene is responsible for a major increase in growth and mature body size. The *hg* gene in the homozygous recessive form causes a rapid increase in post-weaning growth of up to 60%, close to a 30% increase in net energy efficiency, and up to a 5% lower maintenance energy requirement (Bernier et al. 1986, Horvat & Medrano 1995). The efficiency of growth is influenced by this change in energy metabolism.

The origin of the *hg* phenotype is unknown. A few hypotheses have been put forward. The origin could be the result of a spontaneous mutation in a single gene, or it could be caused by a pre-existing allele whose effects are yet to be uncovered in a background of positive growth QTLs generated by selection, or it could be the result of recombination of a cluster of alleles behaving as a single *hg* locus (Horvat & Medrano 1995). By fine mapping the *hg* gene region it would be possible to isolate a smaller genomic segment more suitable for physical mapping and cloning of *hg* (Horvat & Medrano 1995).

By cloning the *hg* region, the *hg* locus could be characterized and its homology determined in livestock species in order to identify a QTL responsible for rapid and efficient growth. If markers were identified within the *hg* region they could be utilized to identify animals with high genetic potential for growth (Horvat & Medrano 1995).

2.2.5.3 Bovine genome map versus ovine genome map

Over the past decade, many genes and other genetic markers have been mapped and the information we possess with regards to livestock genomes has become more abundant. There is a concerted effort to construct gene maps for cattle and sheep using bovine DNA markers, as it has been shown that primers developed from bovine microsatellites can be amplified in sheep DNA (Hediger et al. 1991, Moore et al. 1991, Fries 1993, Vaiman et al. 1994). Moore and colleagues (1991) suggested that in very closely related species, like cattle and sheep, PCR primer pairs designed for use in one species could be used to analyze polymorphic microsatellites in other species. Simultaneous localization of DNA markers on sheep and cattle chromosomes will add directly to each map as well as extend the range of chromosome homology that exists between the species (Drinkwater et al. 1994, Echard et al. 1994). Parsons and colleagues (1998) found that the physical location of the growth hormone receptor (GHR) gene was located on BTA20 and its homolog OAR16, adding to the evidence of conserved synteny and homology between the two species.

Like the cattle genome, the sheep genome also contains a locus responsible for muscular hypertrophy. However, the biology underlying the genes for the phenotype is quite different. In cattle, the cause of increased musculature is hyperplasia, while in sheep the phenotype is caused by hypertrophy of the muscle fibers resulting in leanness and increased feed conversion efficiency. This is referred to as the *Callipyge* (CLPG) locus. In addition, it is subject to an unusual mode of inheritance referred to as polar overdominance, which is similar to a mode of inheritance termed genomic imprinting. In genomic imprinting expression occurs only when the phenotype elicited from a locus is differentially modified by the sex of the parent contributing that particular allele. In polar overdominance only heterozygous individuals having inherited the mutant CLPG allele from their sire and the normal allele from their dam exhibit muscular hypertrophy. The linkage order of microsatellite markers is highly conserved between BTA21 and OAR18 (Moody et al. 1996), therefore the CLPG locus was mapped to ovine chromosome 18 (OAR18) using many BTA21 markers (Cockett et al. 1994). Potential QTLs for growth are associated with BTA21 and thus the genes and markers from OAR18 could be useful in comparative mapping.

Over the past decade, extensive efforts have been made by the international sheep mapping community to develop a useful sheep linkage map. Maddox and associates (2001) have recently developed a medium density linkage map of the sheep genome. The new map comprises 1093 markers. Over 52% of the loci have also been mapped by linkage analysis in cattle. Genomic

coverage by the current sheep map is comparable to that of the available cattle maps.

2.2.5.4 Bovine genome map versus porcine genome map

Although cattle and swine are distantly related, there is still conserved synteny among chromosomes, genes, and other genetic markers. Andersson and associates (1994) found evidence in the porcine genome for QTLs affecting growth and fat deposition on porcine chromosome 4 (SSC4), and QTLs affecting birth weight, early growth, and daily gain on SSC13. The bovine homolog of SSC4 is BTA14, which has been shown to contain QTLs for longissimus muscle area (Stone et al. 1999) and fat deposition traits (Casas et al. 2000). Quantitative trait loci for carcass and meat quality, and growth in swine have been mapped to porcine chromosomes 2, 3, 4, 7, and 8 (Andersson-Eklund et al. 1998, Wang et al. 1998). Should these QTLs map to homologous regions in cattle, the information would be very useful.

Moody and colleagues (1995) utilized a polymorphism in the bovine growth hormone receptor (GHR) gene to localize it to BTA20. The GHR gene in swine had been previously mapped to SSC16 (Chowdhary et al. 1994). Based on the Oxford Grid information that is available from the Mouse Genome Informatics website (www.informatics.jax.org/searches/oxfordgrid.cgi), comparing the arrangement of homologous chromosomes in the bovine and porcine genomes, it is shown that BTA20 and SSC16 are homologous. Connor and associates (1999) were able to map the growth hormone releasing

hormone receptor (GHRH-R) gene to BTA4, which is homologous to SSC18, which also harbors the GHRH-R gene. Information regarding this gene is important as it initiates a response to growth hormone (GH), which relates to differences in growth performance and carcass composition (Connor et al. 1999).

Paszek and colleagues (1999) found a significant QTL effect for post-weaning average daily gain on SSC1; also located on SSC1 is the IGF-1R gene (www.ri.bbsrc.ac.uk/genome_mapping.html). According to the Oxford Grid comparing bovine and porcine chromosomes, SSC1 and BTA21 are partially homologous, and as noted previously, the bovine IGF-1R gene is located on BTA21. Observation of the locus query results obtained from the Roslin Institute website shows that the myostatin gene in swine localizes on SSC15 (www.ri.bbsrc.ac.uk/genome_mapping.html). The bovine homolog to SSC15 is BTA2 (www.informatics.jax.org/searches/oxfordgrid.cgi). Recalling what was stated earlier, the cattle myostatin gene has been mapped to this chromosome. Quantitative trait loci associated with carcass characteristics and growth are closely linked to IGF-1 in cattle. Casas and associates (2000) also reported QTL associated with growth rate located near the IGF-1 locus in swine as well. In swine, IGF-1 is located on SSC5, in cattle, it is located on BTA5, and these chromosomes are homologous.

2.2.6 Candidate genes and positional candidate genes

Candidate genes are genes with a known function, postulated to be responsible for determining a quantitative trait (Elo et al. 1999). If mutated, candidate genes could account for changes in the expression of a trait (Watson et al. 1998). Candidate genes can be linked to another particular locus affecting a desired trait and the two can be transferred together from generation to generation. The candidate gene approach involves the selection of genes with known biological functions relating to the traits of interest. Once QTLs have been mapped to a specific chromosomal segment, the region to search for a possible gene is narrowed and a positional candidate approach can be taken to test the potential of genes as candidate genes (Hetzl et al. 1997). Potential candidate genes are often chosen based on their function, expression or location. Associations between polymorphisms at or near the gene with the traits are tested. The approach is based on close linkage and linkage disequilibrium between loci of the tested candidate gene mutations and the causal mutations.

Candidate genes can be polymorphic; these polymorphisms allow scientists to look for associations across populations. Once an association between the potential candidate gene and the trait is made the resulting marker can potentially be used in breeding programs (de Vries et al. 1998). Potential candidate genes must be tested to determine if they are in fact responsible for phenotypic variation. Hypothetically, constructs containing the candidate gene can be inserted into embryos from phenotypically mutant parents; if the

embryo receives the construct with the correct wild-type gene the individual would appear normal, thus identifying the gene that controls the mutant phenotype (Kappes 1999).

A gene for muscular hypertrophy in sheep (CLPG gene) has been mapped to OAR 18 within a 400kb interval. Four genes have been localized within this region, including DLK 1 (also known as PREF 1), GTL 2 (also known as MEG 3), PEG 11 and MEG 8. Like CLPG they are expressed in skeletal muscle and are subject to parental imprinting (Charlier et al. 2001). From human studies it was postulated that DLK 1 could be a likely candidate gene for the callipyge phenotype in sheep because of its paternal expression, chromosomal location and biological function (Wylie et al. 2000). Linked closely to DLK 1 is GTL 2, which is also a likely candidate though it is expressed maternally, rather than paternally. Fahrenkrug and colleagues (2000) used a comparative mapping approach to identify segments of conserved synteny between HSA 14, BTA 21 and OAR 18. Integrated linkage and physical maps facilitated in the selection of positional candidate genes from the human map. The physically linked genes PREF 1 (DLK 1) and MEG 3 (GTL 2) were mapped to the interval containing the CLPG locus. The group concluded that PREF 1 and MEG 3 were both excellent positional and functional candidate genes. Nevertheless, the interval is likely to contain several other positional/functional candidate genes that will also require assessment by mapping and expression analysis in callipyge sheep.

More recently, Freking and associates (2002) identified a causative mutation for the callipyge phenotype caused by a single base change over a 210kb region surrounding PREF 1 and MEG 3. The SNP was genotyped in multiple families segregating the CLPG locus, providing 100% concordance with animals of known CLPG genotype and was unique to descendants of the founder animal. This discovery marks the starting point for determining the mechanism by which it leads to the marked phenotypic alterations. A better understanding of mammalian protein and adipose accretion, and post-mortem tenderization of muscle tissue should result.

Candidate genes for meat quality have been studied in swine. One example is the heart fatty acid binding protein (H-FABP). This gene contains polymorphisms that are associated with intra-muscular fat (IMF) deposition in certain breeds of swine (de Vries et al. 1998). The H-FABP gene maps to SSC 6. If a homologous gene mapped to this chromosome's syntenic bovine chromosomal region then IMF in cattle could potentially be controlled if the gene acts similarly in both species. Other markers which could be useful as candidate genes for controlling meat quality include myogenin (swine) which increases the number of muscle fibers, and the calpain system and calpastatin gene (beef and sheep) responsible for post-mortem protein turnover, and thus meat tenderness (de Vries et al. 1998, Casas et al. 2000). The growth hormone releasing hormone receptor (GHRH-R) may be a possible candidate gene for controlling growth performance and carcass characteristics in beef cattle (Connor et al. 1999).

Taylor and colleagues (1998) looked at growth hormone 1 (GH1) as a possible candidate gene for QTL effects localized on BTA19 responsible for fat synthesis in cattle. This group used PCR to screen bovine BAC clones containing bovine GH1, FISH to map GH1, development of a microsatellite to test for the presence of a candidate gene, and interval analysis to localize the position of QTLs responsible for growth and carcass traits. The GH1 gene, located at ~73cM on BTA 19, was excluded as a possible candidate gene for the QTLs affecting the ratio of unsaturated to saturated fatty acids in subcutaneous adipose tissues as it was located too far away from the marker. This approach enables researchers to either exclude a gene as being a QTL or can support a candidate gene – QTL relationship through the proximity of the gene to the most likely position of the QTL (Taylor et al. 1998).

A high growth (*hg*) gene was identified in mice in the mid 1990's (Horvat & Medrano 1995). The gene causes a major increase in growth, but does not result in obesity. In mice, insulin-like growth factor 2 (IGF-2) controls prenatal and neonatal growth, growth hormone (GH) and insulin-like growth factor 1 (IGF-1) control postnatal growth, but all of these have been excluded as possible candidate genes for the murine *hg* gene. Horvat and Medrano (1998) isolated the *Raidd/Cradd* gene as the candidate gene for the *hg* gene. These mapping results show that the genomes of *hg* mice represent a naturally occurring knockout of the *Raidd/Cradd* gene, therefore making *Raidd/Cradd* an excellent candidate gene (Horvat & Medrano 1998). This gene is a death adaptor molecule in the apoptotic signaling pathway. Horvat and Medrano

(1998) suggest that the *hg* phenotype may be due to perturbed apoptosis, caused by the lack of function of the *Raidd/Cradd* gene. Molecular cloning of *hg* could allow its functional characterization and enable the isolation and functional studies of a homologous gene in cattle (Horvat & Medrano 1998). This would be useful in designing better strategies for improving post-natal growth in domestic livestock species.

2.2.7 Fine mapping of QTL

The first objective of fine mapping is to construct dense maps of ordered markers within the chromosomal regions harboring genes of interest. Fine mapping of QTL is necessary for providing a useful reference for candidate gene research. It is required due to the lack of both accuracy and precision obtained with conventional techniques for mapping QTL (Riquet et al. 1999). Often the intervals in which the QTL are reported span 20cM to 30cM. Many candidate genes could reside in these large regions, it would be more effective and practical to narrow down the region and thus the number of possible candidate genes. In addition, the support for QTLs is sometimes weak, by narrowing the region in which the QTL is expected to reside, support can be increased and its location is more accurately determined. In recent years new approaches for fine mapping of QTL have been established.

The usefulness of genetic maps in the identification of QTL is a direct function of the overall coverage of the map (Smith et al. 1997). Marker

density of current genetic linkage maps is sufficient for detecting most QTL, but they tend to lack the resolution required for fine mapping and positional cloning of detected QTL within or between breeds (Kappes et al. 1997, Sonstegard et al. 1997). Once more polymorphic microsatellites and candidate genes are mapped, map marker density will increase, and the detection of QTL will be more informative and more accurate.

2.2.7.1 Approaches for fine mapping QTL

The aim of recent genome scan studies was to identify regions containing genes affecting traits of interest. Most of these studies focused on linkage analysis, which utilizes information on recombination events that occur between genetic markers when gametes are formed and transmitted from parent to offspring. However, the low recombination between closely linked markers restricts precision of QTL mapping utilizing linkage analysis (Lund et al. 2003). Generally, the interval within which a QTL is mapped is 10cM to 30cM – which corresponds to ~100 to 300 genes (Glazier et al. 2002). In order to utilize the potential QTL in selective breeding schemes, or to identify functional genes in the region, a higher degree of resolution is required (Lund et al. 1999).

The size of the critical interval must be reduced as much as possible such that the number of candidate genes is modest and functional studies can be undertaken (Glazier et al. 2002). One possibility for fine mapping QTL is to increase the density of the genetic map. Finding and testing new markers at

closer intervals will increase the precision and accuracy of the QTL position and effect. Researchers also share the opinion that there is a need to generate large numbers of progeny; this is applicable to experimental organisms, but nearly impossible for humans and impractical for domestic livestock species (Riquet et al. 1999). Therefore, techniques taking advantage of linkage disequilibrium in populations with limited outbreeding have been employed.

2.2.7.2 Identical by descent haplotype sharing analysis

Linkage disequilibrium (LD) means that the various alleles at two linked loci are not occurring in random associations (Brewer & Sing 1983). The more closely linked two loci are, the greater the number of generations required to bring the population into equilibrium. Linkage disequilibrium is commonly observed in cattle populations, and extends over tens of centimorgans; it has become a widely accepted approach for mapping QTL (Farnir et al. 2000). Linkage disequilibrium can be due to a limited number of common ancestors in a given population and artificial selection over generations. It is particularly useful in identical-by-descent (IBD) QTL mapping using haplotype sharing (deVries et al. 1996, Riquet et al. 1999, Fallin et al. 2001). Co-segregation between a particular genetic marker and quantitative trait loci (QTL) in a population is critical for successfully mapping QTL. Commercial lines of cattle represent semi-closed populations, typically derived from one or a limited number of founders. When selection is applied, common haplotypes originating from common ancestors, harboring QTL of interest, are expected

to carry on and segregate among individuals of commercial breeding lines. Therefore, a direct haplotype comparison can be used to identify the location of a QTL of interest because the chromosome segment housing the QTL is likely to be segregating in the population. This method of fine mapping QTL exploits historic recombinants rather than generating new ones, thus commercial lines can be used. Since it makes use of haplotypes that span two or more loci, both heterozygous and homozygous loci are informative. Fine mapping of QTLs using IBD haplotype sharing analysis for growth as well as for back fat, has been demonstrated in beef cattle (Li et al. 2002, Moore et al. 2003).

2.3 Challenges, Progress, and the Future

Mapping of the human genome was first suggested in the early 1980's, since then there have been many efforts to map the genomes of mice, rats, cats, dogs, and a variety of livestock species (Andersson et al. 1996). With the advent of new technology and new ideas came new challenges; but with these challenges, genomics has made considerable progress. Early challenges, with respect to the bovine genome, included the need to map assigned syntenic groups to actual chromosomes. The utilization of the PCR technique and somatic cell hybrid analysis has lead to the assignment of all syntenic groups to actual chromosomes.

Another early challenge was the development of bovine genetic linkage maps and physical maps. By employing the various techniques, discussed herein, the construction of such maps and their subsequent use has been imperative in determining the location of various genes and other genetic markers. Maps have become easier to construct, and to read, once families in which certain genes were segregating were made available. In addition, DNA markers including polymorphic microsatellites, single nucleotide polymorphisms, single strand conformational polymorphisms, restriction fragment length polymorphisms, and candidate genes have been utilized to aid in the localization of chromosomes. The completion of gene maps has made it possible to select markers from specific genomic locations and use them to systematically screen animal genomes to identify loci that are associated with traits of economic importance.

According to Kappes (1999), the resolution of comparative maps is still very low, thus it can be difficult to determine the homologous regions on those maps. Ma and associates (1998) discuss the comparative mapping by annotation and sequence similarity (COMPASS) method which can be used in a highly selective and directed manner to fill in gaps in RH and comparative maps. Comparative mapping information will become more useful as more genes are identified for different traits, and more genes are added to livestock maps in the form of expressed sequence tags (ESTs).

Sequence tagged sites are short (200 to 500 base pairs) DNA sequences that have a single occurrence in a genome and whose location and base sequence are known (<http://www.books.md/S/dic/sequencetaggedsite.php>). They are detectable by PCR, thus are useful for localizing and orienting mapping and sequence data reported from many different laboratories and also serve as landmarks on the developing physical maps of genomes. Expressed sequence tags are sequence tagged sites derived from cDNAs; they are unique sequences from a known location, easily amplified using the PCR method (Dietz et al. 1992). The sequencing and mapping of additional cattle ESTs and sequence tagged sites will allow the construction of more highly defined comparative maps, creating a powerful tool to aid in identification of genes affecting economically important traits and contributing to the understanding of mammalian chromosome evolution. As more markers become available, their usefulness between species will be determined.

The resolution of livestock comparative maps is a major obstacle in identifying candidate genes. Of the thousands of genes existing in the cattle genome, only 200 are on the ordered genetic map of cattle (Ma et al. 1996, Barendse et al. 1997, Kappes et al. 1997). However, the recent development of radiation hybrid (RH) panels of whole genome cattle-hamster cells has fueled the continued advancement in cattle genomics and comparative mapping. Despite the creation of the RH panel, mapping on it is not yet progressing rapidly because of limited homologous DNA sequence information (Ozawa et al. 2000).

Ma and associates (1998) proposed the COMPASS strategy – comparative mapping by annotation and sequence similarity. Provided there is detailed sequence and mapping data for a reference species and adequate existing comparative mapping information for the target species COMPASS allows the prediction of map location of a randomly chosen DNA segment (Ma et al. 1998). Although the COMPASS method is accurate in assigning the location for bovine genes, there are sources of error. The existence of paralogs and pseudogenes creates difficulty in elucidating the true comparative chromosome organization among species. Secondly, the low resolution and accuracy of comparative maps limits the accuracy of COMPASS (Ozawa et al. 2000). As map resolution and accuracy increase, and the mapping of additional ESTs occurs, combined with the use of higher resolution RH panels, the accuracy of COMPASS's prediction should improve (Ozawa et al. 2000).

Band and colleagues (2000) constructed a cattle-human whole genome comparative map using radiation hybrid mapping coupled with EST sequencing, database mining for unmapped cattle genes, and a predictive bioinformatics approach (COMPASS). The group placed 768 genes and 319 microsatellites on the map. The COMPASS mapping tool was shown to be 95% accurate in predicting cattle chromosome location from random sequence data. The comparative map generated will be a cornerstone for elucidating mammalian chromosome phylogeny and identification of genes of agricultural importance.

Quantitative traits represent the greatest challenge for molecular genetic analysis because of the variation due to the interaction of many genes and the environment (Hetzel et al. 1997). The challenge in the early 1990's was to identify allelic variation in QTLs when the QTLs themselves could not be detected directly. The proposed method for this determination was the use of polymorphic marker loci segregating in large families of paternal half-sibs (Beever et al. 1990).

Beever and associates (1990) hypothesized that if a parent was heterozygous at a marker locus then half-sibs could be divided into two distinct groups and if the sire was also heterozygous at a closely linked QTL then offspring would differ with respect to the quantitative trait. Since the proposed hypothesis, much work has been done with large paternal half-sib families. The goal now is to use more, larger families from different populations to map QTLs having effects on economic traits, especially growth and carcass characteristics (Kappes 1999). In addition, as the number of polymorphic microsatellites available increases, mapping QTLs will be less difficult, and more informative.

Another challenge regarding the search for QTLs is the fact that it is not known whether mapped QTLs are segregating within other herds of the same type, and different types, versus those that have been tested. Therefore, there is a great need to evaluate markers in industry relevant breeds and breed crosses (Hetzel et al. 1997). The information regarding QTLs will be most

informative when it can be applied to commercial herds. The primary goal of QTL research is the ability to identify QTLs with varying degrees of effect, develop gene marker tests and then incorporate them into genetic evaluation schemes and breeding programs at the commercial level (Hetzl et al. 1997). This will provide us with the opportunity to evaluate estimated breeding values (EBVs) based on molecular genetics techniques, versus traditional performance records.

Once a gene is isolated, a gene marker test can be developed based on the allelic variant responsible for a particular effect and these tests can be utilized without the knowledge of an individual's pedigree (Hetzl et al. 1997). Presently, our ability to determine an animal's genetic merit for traits difficult to measure in live animals prior to processing and product distribution is limited. Usually, the genetic merit of an individual, is based on expected progeny differences (EPDs) or estimated breeding values (EBVs), obtained via performance evaluation of sires and their progeny. Progress needs to be made in the direction of accurate prediction of genetic merit in the early stages of production. For cattle populations in which QTLs, having major effects, are segregating, calves could be sorted early on and their genotypes matched with production targets (Stone et al. 1999). Relative to this would be matching genotypes to finishing programs that would yield products to satisfy consumer demands.

The localization of genes responsible for the variation in growth and carcass traits will be very useful because the mapping of such loci will eventually enable us to characterize or identify the genes. This will improve our understanding of economically important traits and their underlying biology. The future will likely see the use of a great amount of marker assisted selection (MAS). Information gathered at the DNA level will be important in fixing specific major genes, either for or against a particular trait we are already selecting for (Charlier et al. 1995, Charlier et al. 1996, de Vries et al. 1998). By fixing such genes in a population, selection accuracy and selection response will be increased. In addition to fixing genes from current populations, if specific gene markers could be isolated in past resource germplasm these genes could be incorporated into present populations inferior in that trait (Beever et al. 1990). Before MAS can be utilized efficiently, fine mapping of QTLs, and positional cloning of these QTLs, and the genes associated with them, must take place. Most QTLs thus far have been mapped to regions greater than 30cM; therefore, they need to be fine mapped to narrow down the region.

Individuals with a desired QTL could be retained in the herd and used for breeding purposes, keeping the alleles of interest in the population. Once a QTL for a particular growth trait has been identified, that trait could be continually selected for and introgressed into cattle herds via breeding with individuals known to carry the haplotype responsible for the trait. Typically, high gain is associated with high birth weight. However, low birth weight

could be coupled with high pre- and/or post-weaning rate of gain. Post-weaning growth could be greatly enhanced if a rapid genetic test for identifying significant growth QTLs was developed. In addition, this would also increase the potential rate of genetic progress for economically important traits. The tests for DNA typing that will be developed, as a result of this study, should be validated using commercial herds. These tests will provide the beef cattle industry with the information required to select for higher growth potential in herds; or as a tool to optimize finishing regimes and produce more uniform consumer products, by grouping cattle according to their expected growth rates.

The mapping of quantitative trait loci in the bovine genome, by molecular genetics techniques, will provide the necessary information required to isolate and sequence genes and to analyze their function. Determination of the chromosomal location of genes affecting specific traits will be useful in understanding the biology underlying genetic variation in many species. If a QTL affecting growth, for example, is isolated in one species, it can potentially be mapped, isolated, and eventually analyzed in another species, based on homology.

Genetic tests could be developed for DNA typing to validate QTLs in commercial herds. Currently, QTLs have been isolated primarily in experimental populations; ones in which large phenotypic differences are obvious, such as the crossing of *Bos indicus* and *Bos taurus* cattle. The goal is

to verify QTLs segregating in other families of the same breeds tested experimentally, other breeds, and other breed crosses, in particular common breeds in the commercial beef cattle industry.

Most allelic segregation is determined by the transmission of paternal genes from generation to generation, by utilizing large half-sib families with the same sire and many sons. As new techniques are employed and more cattle families are utilized, research will be able to focus on maternal segregation of genes as well.

Most quantitative traits are economically significant in the livestock industry. These include birth weight, pre-weaning average daily gain, post-weaning average daily gain on feed and other carcass traits in the beef cattle industry. By locating the QTLs responsible for growth, and the chromosomes on which they reside, we will have the potential opportunity to control the traits affected. Genetic tests will provide information to producers that will allow them to place calves in appropriate finishing programs so that all animals are reaching their genetic potential, efficiency is maximized, and consumers are satisfied.

Knott and Haley (1992) summarized the importance of QTL mapping of traits of economic importance. First, it provides knowledge regarding individual gene actions and interactions. Second, marker information could be used directly to improve breeding value estimations and marker assisted selection and introgression could be effective means of introgressing a few genes of

value from one breed or line to another for improving selection responses within a breed. Third, the mapping of a QTL would provide a route for the eventual cloning of a locus. Mapping of a QTL to a known chromosomal location is a vital step, but more must happen. The region must then be saturated with markers, tested in many individuals, and fine mapped in order to determine the underlying causative gene or mutation associated with the resulting phenotype. This phenotype can then be selected for in future generations.

The myostatin gene has been mapped to BTA 2. The gene is associated with muscular hypertrophy in cattle. Cattle with two inactive myostatin alleles exhibit the double muscled phenotype, and appear more heavily muscled than animals inheriting normal alleles (Charlier et al. 1995, Casas et al. 1997, Grobet et al. 1997, Sonstegard et al. 1997). Many growth factor genes and QTLs for growth and carcass traits have been mapped to BTA6 (Teres et al. 1996, Casas et al. 2000). On BTA14, there are QTL for longissimus muscle area (Stone et al. 1999) and fat deposition (Casas et al. 2000). Hediger and colleagues (1990) mapped the bovine growth hormone (GH) locus to BTA19 by in situ hybridization. The GH gene was also placed on BTA19 by Womack and associates (1989) by radiation hybrid mapping. The principle objective of work with BTA19 and the GH gene is to enhance growth rate, efficiency of growth, and carcass characteristics (Rodrigues et al. 1998, Taylor et al. 1998). Bovine chromosome 21 is homologous to OAR18 and both contain the IGF-1R gene, which is important in growth (Moody et al.

1996). Finally, BTA23 has a QTL for live weight (Elo et al. 1999); it also contains the major histocompatibility complex, which has been shown to be associated with growth traits (Beever et al. 1990).

The next stage of research will be to fine map QTL regions associated with growth, which will be possible without dependence on pedigree information. By fine mapping QTLs we will be able to select and test for some candidate genes with known position, and the subsequent development of markers that will directly identify growth traits. Chromosomal regions containing such QTL will be fine mapped in order to develop linked markers that will be of critical significance in MAS.

In this study, a number of chromosomes previously reported to contain growth QTLs were screened using microsatellite markers flanking the QTL regions. The chromosomes chosen in this study, based on previously reported QTL for growth are BTA2, BTA6, BTA14, BTA19, BTA21 and BTA23. Microsatellites were evenly spaced at approximately 10cM intervals along each chromosome. Regions in which QTLs were identified were further narrowed down for fine mapping.

3. MATERIALS AND METHODS

3.1 Animals , DNA samples and phenotypic data

Beefbooster Incorporated, Canada was the company responsible for breeding the cattle used in this study. The company offers cattlemen five specialized hybrid seedstock strains: three maternal strains (M1, M2 and M4) to sire top replacement heifers and cows, one heifer strain (M3) to ensure ease of calving for heifers and one terminal strain (Tx) for producing offspring that will grow rapidly.

Beefbooster is an organization dedicated to producing high quality, functional seedstock to enhance profitability of commercial beef production. Beefbooster is recognized as the top supplier of hybrid bulls in Western Canada and the American Northwest. Bulls are raised and tested under commercial conditions similar to many customers' environments. The only special management of seedstock herds is that required to measure and weigh cows and calves. Each of the specialized strains was developed to meet the needs of commercial beef production.

In the spring of 1998, male calves from the five strains were identified and calf birth weights at or near birth, as well as birth date, were recorded. A 10-mL blood sample was collected by venipuncture from each male calf, and the potential sires. The DNA from each blood sample was extracted and kept for

later parentage identification. Sire identification was carried out by the Saskatchewan Research Council, Canada using DNA microsatellite markers.

Meanwhile, cows and calves were placed on tame pastures from May through to mid-September and October. In the fall of 1998, the calves were weaned and weighed between September 16 and October 20. Over one-third (38.6%) of the bull calves with the lowest pre-weaning gain were then culled. The remaining male calves were placed in feedlot pens for post-weaning performance testing. Briefly, animals were first placed in one of the feedlot pens where they were adjusted to diet and environment over 21 to 29 days before the test. During the adjustment period, animals were fed a diet consisting of 50.5% barley silage, 15.0% chopped hay, 21.1% rolled barley grain, 10.0% wheat mill run pellets and 3.4% calf supplement. After the adjustment, animals were placed on a 120-day growth performance test. During the first 17 days of testing, the animals were fed the same diet as in the adjustment period, followed by 22 days of testing in which they were fed a diet consisting of 72.0% barley silage, 5.2% rolled barley grain, 20.0% wheat mill run pellets and 2.8% calf supplement. During the final 81 days of testing, the animals were fed a diet consisting of 87.5% barley silage, 10.1% wheat mill run pellets and 2.4% calf supplement.

The DNA sample set, utilized in this study, was created using samples from the M1 Line of Beefbooster cattle. These cattle are an intermediate framed strain developed from an Angus base and have been selected based on a

selection index that is in favor of lower birth weight, higher pre-weaning gain and higher post-weaning gain of the calf, and the dam's excellent mothering ability and a sound udder (MacNeil and Newman, 1994). The sample set utilized in the study, contained 12 sire samples and 176 male calf samples (from those 12 selected sires). Selection of the samples was based on the number of offspring the sire had, as this influences the validity and significance of the findings generated in the analysis of the data. The DNA samples for genotyping were run on agarose gels to determine the approximate concentration of DNA, then diluted to a working concentration of 0.3125ng/ul for use in PCR.

Phenotypic data (including birth weight, pre-weaning average daily gain, and post-weaning average daily gain on feed) was provided by Beefbooster. The performance of every animal was carefully measured and data was analyzed with statistical techniques to obtain the best possible predictions of genetic merit for every animal. The pre-weaning average daily gain was calculated by the difference between weaning weight and birth weight, dividing by days between weaning date and birth date. The average daily gain on feed was then calculated by the difference between weight when the test started and weight when the test ended, dividing by the days of test.

3.2 **Genetic markers and genotyping**

Using the Meat Animal Research Center (MARC) database made available on the World Wide Web (<http://www.marc.usda.gov/genome/genome.html>) a genetic map of each selected chromosome, with all currently mapped genes and microsatellites presented on it, was located. Each chromosome map provided information on the location, DNA sequence, the marker size, the number of alleles, the annealing temperature for PCR reactions, and the scoring difficulty for each marker selected. According to the MARC database, the sizes of the bovine chromosomes 2, 6, 14, 19, 21 and 23 are 120.4cM, 125.6cM, 86.7cM, 99.5cM, 88.6cM and 67.2cM, respectively.

Seventy-one genetic markers were chosen (8 to 17 markers per chromosome) to span the six chromosomes under investigation. A table indicating each chromosome's genetic markers with primer sequence, size, number of alleles and marker type data is presented in the Appendix (Chapter 7). The genetic markers chosen were approximately evenly spaced along each chromosome. The markers included TGLA 44, TGLA 431, TEXAN 2, CSSM 42, BMS 1300, ILSTS 098, BMS 1837, BMS 1866, TEXAN 4, TEXAN 5, BMS 356, BM 2113 and OARFCB 11 from bovine chromosome 2. On bovine chromosome 6 – INRA 133, ILSTS 090, BM 1329, BMS 2508, BMS 382, BMS 1242, BM 4322, BMS 470, BMS 360, BM 4528, RM 028, OAREL 03, ILSTS 087, BM 1236, BMS 2460, ETH 8 and BMC 4203 were selected. From bovine chromosome 14 – DGAT 1, CSSM 66, BMS 1747, TG, BMS

1678, BMS 1941, BMC 1207, BM 1577, BMS 108, BMS 1899 and RM 137 were chosen. Markers BM 6000, BMS 745, CSSME 70, RM 222, BP 20, ILSTS 014, BMS 2503, BM 2389, BM 17132, RM 099, CSSM 65, BMS 1069, RM 388 and BMS 601 were chosen from bovine chromosome 19. On bovine chromosome 21 – BM 8115, BMS 1117, AGLA 233, BP 33, BMS 2815, ILSTS 092, ILSTS 054 and BMS 2382 were selected. Finally, INRA 064, RM 033, BM 1815, BM 1258, BMS 468, RM 185, CSSM 24 and BM 1443 were chosen from bovine chromosome 23. With the exception of DGAT 1 and TG, all markers were microsatellites while DGAT-1 is a single nucleotide polymorphic (SNP) marker and TG is a restriction fragment length polymorphic (RFLP) marker. The primers for genotyping of the markers were synthesized by Invitrogen Life Technologies, Canada, Sigma-Genosys, USA, New England BioLabs Inc., USA, Applied Biosystems, USA and Lethbridge Research Center, Canada.

3.3 Experimental procedure

3.3.1 Radioactive PCR and gel method

The genotyping of some of the genetic markers from BTA 6 (including BM 1329, BM 3026, BMS 690, BM 4322, BMS 360, BM 4528, RM 028, OAREL 03, ILSTS 087 and BMS 2460) and some of the genetic markers from BTA 19 (including BM 6000, BMS 745, CSSME 70, RM 222, BP 20, ILSTS 014, BM 2389, CSSM 65, BMS 1069, RM 388 and BMS 601), was performed using a

radioactive ($[\alpha\text{-}^{33}\text{P}]\text{dCTP}$) polymerase chain reaction (PCR). The purpose of the PCR is to amplify a certain sequence of DNA many times; in these experiments, the sequences that were amplified were those of the genetic markers chosen for analysis. Radiolabeling of one of the DNA bases (in this case dCTP) allows the banding pattern of the marker to be seen once the gel is developed on a sheet of scientific imaging film. Once the markers were determined to be working, a $[\alpha\text{-}^{33}\text{P}]\text{dCTP}$ labeled PCR was run using each marker and the complete set of DNA samples.

The protocol for performing the radioactive PCR for 100 samples is as follows: in a 1.5mL eppendorf tube mix 1355.0 μL sterile water, 250.0 μL 10X PCR buffer, 50.0 μL dNTPs (10mM dATP, dGTP, dTTP, 0.75mM dCTP), 25.0 μL $[\alpha\text{-}^{33}\text{P}]\text{dCTP}$ (10mCi/mL), 200.0 μL MgCl_2 (25mM), 50.0 μL forward primer (20 $\mu\text{M}/\mu\text{L}$) and 50.0 μL reverse primer (20 $\mu\text{M}/\mu\text{L}$), microcentrifuge for 10seconds and add 50.0 μL *Taq* polymerase (5units/ μL), finger flick to mix. Add 5 μL of each DNA sample to separate 0.2mL PCR tubes, add 20.0 μL of PCR master mix to each 0.2mL PCR tube and close the lids. Arrange tubes in thermocycler (BioRad iCycler®) and set the instrument to run the touchdown program. When the PCR is finished, add 50.0 μL of stop buffer (containing 95% (v/v) formamide, 0.5M EDTA, sterile water, bromophenol blue and xylene cyanole FF) to each PCR tube and store the samples at 4°C (degrees centigrade).

The first step of the touchdown polymerase chain reaction (PCR) is to heat the tubes to 94°C for 3minutes. This initial denaturation step ensures the splitting of the DNA template, in this case the genetic marker. The polymerase chain reaction can be divided into three basic parts, carried out in one tube, at different temperatures. The first phase of the process separates the two DNA strands in the double helix by heating the sample to 94°C for 30seconds.

However, the primers cannot bind to the DNA template strands at such a high temperature, so the tube is cooled to either 66°C or 60°C. At this temperature, the primers are able to anneal to the ends of the DNA strands. This also takes about 30seconds. The temperature for annealing is dependent on the genetic markers being synthesized. In this particular study, the annealing temperatures were such that either a 66°C touchdown PCR or a, 60°C touchdown PCR were appropriate.

The final phase of the reaction is to make a complete copy of the genetic marker template. Since the *Taq* polymerase works best at around 70°C (the temperature of the thermal habitats in which it survives range from 50°C to 80°C (<http://www.bact.wisc.edu/Bact303/b27>)), the temperature of the tube is raised. This particular experiment increased the temperature for synthesis to 72°C for 30seconds.

The *Taq* polymerase adds nucleotides, provided as dNTPs, to the primer and eventually makes a complementary copy of the template. If the template contains an A nucleotide, the enzyme adds on a T nucleotide to the primer. If

the template contains a G, it adds a C to the new chain, and so on to the end of the DNA strand. This completes one PCR cycle.

The three steps in the PCR - the separation of the strands (melting), annealing the primer to the template (annealing), and the synthesis of new strands (elongation) – are carried out in the same tube. At the end of a cycle, each piece of DNA in the tube has been duplicated.

This particular protocol utilizes two initial cycles with an annealing temperature of either 66°C or 60°C. The higher temperature results in the generation of more specific PCR products. Following these two cycles, the samples are subjected to 12 cycles in which there is a 0.5°C decrease in annealing temperature with each cycle. In the 12th cycle, the annealing temperature is either 60°C or 54°C. Once this annealing temperature has been reached, another 30 cycles, with an annealing temperature of either 60°C or 54°C, are performed. The annealing temperature is decreased in the 12 cycles to increase product yield. At the higher temperature, yield is sacrificed. Therefore, by starting with the higher temperature, we increase specificity, and by decreasing the temperature in subsequent cycles, we increase yield. The use of a touchdown program utilizing the temperatures between 66°C and 60°C, or between 60°C and 54°C was appropriate for all of the genetic markers utilized in this study.

The cycle is repeated 30 times with an annealing temperature of either 60°C or 54°C. Each newly synthesized DNA piece can act as a new template, so after

30 cycles, theoretically, 1 billion copies of a single piece of DNA can be produced. PCR is valuable to researchers because it allows them to multiply unique regions of DNA so they can be detected in large genomes.

At the end of the 30cycles, the temperature is maintained at 72°C to allow the reaction to go to completion. The final step is to store the products at 4°C. Addition of the stop buffer dilutes the sample, prevents degradation of the DNA, and incorporates dyes that function as markers when running the gel, which are useful in determining how long electrophoresis should take place.

The PCR products were then run on a 0.4mm thick Long Ranger® 5% (w/v) (7M Urea) polyacrylamide gel. The gel was cast between two glass plates, which were part of a BioRad Sequi-Gen® GT nucleic acid sequencing cell apparatus. Using this system, constant power is applied to the gel apparatus unit. This results in the heating of the gel. Once the gel reached approximately 50°C, 5.0µL of each denatured PCR product (samples denatured in BioRad iCycler® for 3minutes at 95°C then immediately placed on ice) was loaded into separate wells. The samples were run on the gel for approximately two hours; then the buffer chambers of the unit were drained (1X TBE buffer). The glass plates were removed from the instrument and the gel was mounted onto a sheet of 3mm thick Whatman® chromatography paper and covered with Saran wrap. It was subsequently dried in a gel dryer (BioRad Model 1125B or BioRad Model 583) until the paper was no longer sticky to the touch. Upon drying, the Saran wrap was removed. A sheet of

Kodak Biomax MR scientific imaging film was placed on top of the gel in an autoradiography cassette, in a darkroom. After 2 to 5 days, the film was removed from the cassette and developed, in a darkroom using a Kodak M35A X-OMAT processor. The products were detected by autoradiography.

3.3.2 Dye labeled PCR and gel method

Additional genetic markers from BTA 6 (including INRA 133, BMS 2508, BMS 382, BMS 1242, BMS 470, BM 4621, BM 1236 and BMC 4203) and additional genetic markers from BTA 19 (including BMS 2503, BM 17132 and RM 099), and all of the genetic markers from BTA 2, BTA 14, BTA 21 and BTA 23 were typed for the 176 calves and their 12 sires using an ABI PRISM™ 377 DNA Sequencer. Each genetic marker's reverse primer was labeled with one of three fluorescent dyes – TET, HEX or 6-FAM. The TET label causes the bands to appear green, while HEX labels the bands yellow, and 6-FAM bands are blue.

A PCR similar to that used in the radioactive method was done for all samples with each primer to be typed. The radioactive PCR utilized a 25µL reaction, while the dye-labeled PCR utilized a 15µL reaction. With the exception of the fluorescent dye-labeled primers and amounts of other ingredients reduced proportionally, the PCR conditions are the same for the ABI PRISM™ 377 method as was stated for the ³³P-labeled method.

Upon completion of the PCR, a 1.0µL aliquot was withdrawn from each sample and loading mix, containing deionized formamide (2.5µL/reaction)

and blue loading buffer (0.5µL/reaction), was added to the PCR product aliquot, and the samples were then stored at 4°C. The purpose of the loading mix is the same as that of the stop buffer used in the radioactive method. A GeneScan-350 (Tamra) size standard plate was prepared at least 1 hour before samples were to be run on the instrument. A single plate well contained 0.35µL of GS-350 (Tamra) size standard, 0.15µL of loading buffer and 0.15µL of sterile water. The plate was covered with parafilm and a needle was used to poke a hole through the parafilm at the top of each well. The plate was spun down in a centrifuge (Beckman Coulter® TJ-25 centrifuge) to ensure all of the size standard mixture was in the bottom of the well. The plate was then covered with tinfoil; the size standard is light sensitive and will degrade if not kept in the dark. The size standard plate was dried out in a 37°C oven (Precision Scientific Thelco Model 6) for 1 hour or left at room temperature over night. A 1.0µL aliquot of PCR/loading mix of each sample was transferred to the dried size standard plate and spun in the centrifuge (Beckman Coulter® TJ-25 centrifuge) to ensure all liquid was at the bottom of each well. The plate, now containing: size standard, loading mix and PCR product, was left sitting at room temperature, covered in tinfoil, for one hour to allow the size standard to go into solution. Samples were then denatured for 3minutes at 95°C (BioRad iCycler thermocycler), immediately placed on ice and then loaded onto the gel.

The ABI PRISM™ 377 DNA Sequencer utilizes a 0.2mm thick Long Ranger® 5% (w/v) (7M Urea) polyacrylamide gel cast between two glass

plates. The instrument is designed for analyzing fluorescently-labeled DNA fragments by gel electrophoresis. Once the samples are loaded, voltage is applied which causes fragments to electrophorese through the gel and separate according to size. At the lower end of the gel, a laser beam continuously scans across the gel exciting the fluorescent dyes attached to the fragments. Data collection software collects the light intensities information from a charge coupled device (CCD) camera and at the end of collection the analysis software (ABI PRISM GeneScan® Analysis software) is used to process, analyze, and translate the collected data into fragment sizing information. More detailed information is available in ABI PRISM™ 377 DNA Sequencer: For Sequencing and GeneScan® Analysis Software Applications User's Manual, Applied Biosystems, A Division of Perkin-Elmer, USA.

3.4 Genotyping

The one hundred and seventy-six calves and their 12 sires (9 to 30 calves of each sire) of the M1 line were genotyped using seventy-one genetic markers chosen from BTA 2, 6, 14, 19, 21 and 23 (8 to 16 markers from each chromosome). As described above, the thirteen markers chosen from BTA 2 cover 99.3% of the chromosome. Seventeen markers were selected on BTA 6; they spanned 83.6% of the chromosome. On BTA 14, eleven markers were chosen, encompassing 70.0% of the chromosome. Fourteen markers covered 95.1% of BTA 19. There were eight markers chosen from BTA 21, spanning

98.9% of the chromosome. Eight markers selected on BTA 23 encompassed 99.9% of the chromosome.

3.4.1 Genotyping using ^{33}P

Once the scientific imaging film was developed, each genetic marker was scored independently by two individuals. The scoring process involved a visual analysis of each developed film. The alleles that have been inherited by the calf appear as very dark bands, with a distinct pattern, on the film. Alleles were identified by their banding pattern and each calf sample was assigned two alleles for each genetic marker genotyped. Sire samples were also assigned two alleles and calves were checked against sires to ensure inheritance of one allele.

3.4.2 Genotyping using ABI PRISMTM fluorescent dyes

For the ABI PRISMTM GeneScan Analysis method, results data including: dye color, sample fragment size and quantitative data, and sample information is collected in GeneScan[®] files. The results of this fragment analysis are imported into and analyzed by Genotyper[®] 2.5.2 software. The user sets up bins, or categories, which determine how the peaks will be labeled on an electropherogram. Each bin represents one allele of a particular size.

Since each sample contains a size standard, the software is able to label peaks relative to the size of the fragments. For example, if the microsatellite under investigation is a dinucleotide repeat, bins are created such that each bin is two base pairs in size, and has a tolerance level of ± 0.50 base pair. The

highest peak falling within that two base pair range is assigned that specific allele. A category is defined by the highest peak in a given size range for a particular dye. The software is then able to analyze each sample's fragments and displays them as labeled peaks in an electropherogram (Figure 1). The software screens out peaks resulting from PCR-related artifact fragments (stutter) detected during electrophoresis via filtering parameters set by the user.

3.4.3 Genotyping of DGAT 1 and TG

Using an ABI PRISMTM 7700 sequence detector the genotyping of the DGAT 1 gene-specific SNP was carried out based on allele discrimination using the 5' nuclease assay (Applied Biosystems). Briefly, a forward primer (5'cgc ttg ctc gta gct ttg g 3') and a reverse primer (5'ccg cgg tag gtc agg ttg t 3') were designed to amplify the dinucleotide substitution (AA/GC) region at the beginning of exon VIII based on the *Bos taurus* diacylglycerol acyltransferase 1 gene sequence (GenBank Acc. No. AY065621). Two fluorogenic probes were also designed to target the two alleles, with FAMTM reporter dye for allele Q (AA) and VICTM reporter dye for allele q (GC). The sequences of the probes for allele Q and allele q detection were 5' ccg ttg gcc ttc tta 3' and 5' ccg ttg gcc gcc tt 3', respectively. A perfect match of a probe sequence, to the target sequence, results in the cleavage and release of the reporter dye. Thus, substantial increase in either FAM or VIC dye fluorescence indicates homozygosity for the FAM-specific allele (allele Q) or for the VIC-specific allele (allele q). An increase in both signals indicates heterozygosity.

The genotyping of TG was carried out as described by Barendse (1999). Briefly, the genomic DNA was amplified using primers TG5D1 (5' gtg aaa atc ttg tgg agg ctg ta 3') and TG5U2 (5' ggg gat gac tac gag tat gac tg 3'). The PCR products were digested using Mbol (cleaves at 'GATC' site) (New England Biolabs) by incubating the samples at 37°C for one hour. The fragments were separated on 3% agarose gels (Sigma) by electrophoresis, with 1xTBE buffer; and stained using ethidium bromide. The genotype of each animal was determined based on the fragment profile, with allele '2' being cut and allele '3' uncut.

3.5 Haplotype identification

Each calf will inherit two alleles for each genetic marker genotyped. One allele is inherited from its dam and one allele is inherited from its sire. The calf can inherit two homozygous alleles, or the alleles may be heterozygous. For each calf, alleles of each locus, contributed by the sire and by the dam, were identified. At least one of each calf's two alleles must match one of its sire's two alleles. This common allele has been inherited from the sire, by the calf. With this data, it is possible to determine which allele was inherited from the sire and which was inherited from the dam. Sometimes, due to experimental error, the calf's alleles do not match the sire's alleles, or no alleles are detected – these samples are deleted from analysis.

The genetic markers are arranged in the proper order, i.e. the order in which they have been previously mapped to their respective chromosome according to the USDA website, in a spreadsheet. From this spreadsheet, the haplotype (allele linkage phases) of each individual calf can be identified. The haplotype is determined by combining the allele of one locus, inherited from the sire, with the allele from the adjacent locus, also inherited from the sire. For loci BM1329 and BMS 2508, the haplotype 5-102 represents a section of the chromosome having allele 5 at BM 1329 (BM 1329-5) and allele 102 at BMS 2508 (BMS 2508-102). Haplotypes of two adjacent loci occurring at a frequency of more than 8% were used in further analysis of associations between a haplotype and the growth traits under investigation. Haplotypes that extended across more than two loci were not included in the final analysis because of their low frequencies.

3.6 Statistical analysis

The theoretical basis for identical-by-descent haplotype sharing analysis has been described in detail elsewhere (Donnelly 1983, Lander and Botstein 1986, de Vries et al. 1996). Briefly, two individuals with a given relationship are assumed have some segments of chromosomes in common, due to descent from a common ancestor. The expectation for haplotype sharing to occur is dependent upon a limited number of introductions of a gene into a population from a small number of predecessors to a larger population (de Vries et al.

1996). It is expected that individuals within a semi-closed population, such as a commercial line of beef cattle, are derived from one or a limited number of founders. Thus, common haplotypes from these common ancestors are expected to carry on and segregate in further generations, particularly when selection is applied. Selection in favor of a particular trait increases the percentage of the haplotype harboring genes of importance, this makes identical by descent (IBD) mapping more feasible.

The IBD haplotype sharing method takes advantage of linkage disequilibrium, because the population under investigation has limited outbreeding, and arose from a limited number of founders. Therefore, it is possible to identify chromosome segments housing QTL via direct haplotype comparison. Haplotype sharing analysis exploits historical recombinant rather than generating new ones.

Once the haplotypes were established along each chromosome, the data was analyzed using the general linear model (GLM) procedure of SAS Version 8 (SAS Inst. Inc., Cary, NC). The GLM procedure tests the association between a given haplotype and birth weight (BWT), pre-weaning average daily gain (PWADG) or average daily gain on feed (ADGF). The summary statistics of the three growth traits analyzed for each chromosome are summarized in Table 1. The linear model was: $Y_{ijk} = \mu + T_i + H_j + (TH)_{ij} + \beta(A_{ijk} - \bar{A}_{..}) + E_{ijk}$, where Y_{ijk} = the observation of the i^{th} haplotype under the j^{th} herd effect and the k^{th} dam age effect, μ = overall experimental mean, T_i = haplotype

effect, taking 1 when the individual has the haplotype and 0 when the individual is without the haplotype, H_j = herd effect, taking 1 or 2 (2 herds were used), TH_{ij} = the interaction between the haplotype effect and the herd effect, $\beta(A_{ijk} - \bar{A}_{..})$ = dam age effect as a covariate, and E_{ijk} = residual error.

Type III sum of squares was used in all F -tests. Haplotype effect and herd effect were treated as fixed effects, while dam age, was treated as a covariate. The individuals with the haplotype in question were compared to the individuals without the haplotype in question. Animals with an uncertain haplotype were considered as missing values and were deleted from the analysis. Individuals carrying one copy of the haplotype or two copies of the haplotype were treated the same because only a few animals carry two copies of the haplotype. The GLM analysis provided the necessary information to determine whether there is a QTL effect present in association with particular haplotypes, and thus the approximate location of the determined QTL.

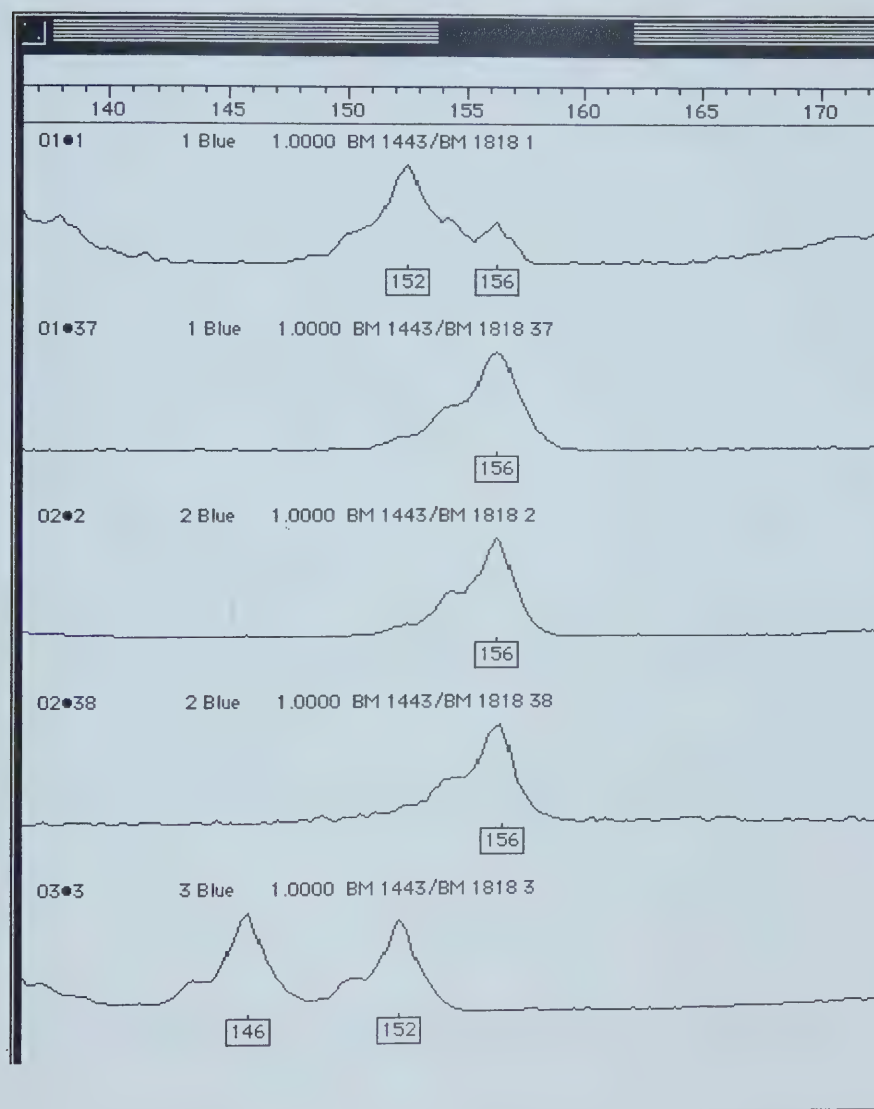
The comparison-wise and chromosome-wise threshold P -values were generated empirically from a modified version of the permutation method outlined by Churchill and Doerge (1994) and as described by Li and associates (2002) for the three growth traits in the M1 line. Briefly, individuals were indexed from 1 to n . Data from the three growth traits was combined, randomly shuffled and also indexed from 1 to n ; it was then assigned back to the individual with the same index. Randomly shuffled data was analyzed again for the common haplotypes along each of BTA 2, 6, 14,

19, 21 and 23. Corresponding P-values were stored and the lowest P-value among the three traits was recorded; the process of shuffling was repeated 1000 times. Comparison-wise threshold P-value for a given haplotype was calculated by choosing the $100(1-\alpha)$ percentile (α is the Type I error, in this case we chose to use 0.05%) of the corresponding P-value distribution of the haplotype. This same method was used in determining the chromosome-wise threshold P-value, by choosing the $100(1-\alpha)$ percentile (chose $\alpha = 0.10$) of the corresponding P-value distribution of all haplotypes along the chromosome.

Table 1 Summary statistics of birth weight, pre-weaning average daily gain and average daily gain on feed in M1 line of *Bos taurus* from Beefbooster, Inc.

TRAIT	MEAN	RANGE	S.D.
BWT (Kg)	41.35	27.24 – 52.66	4.48
PWADG (Kg)	1.15	0.87 – 1.53	0.11
ADGF (Kg)	1.33	0.48 – 1.94	0.18

Figure 1 Example of an electropherogram for locus BM1443 (dye labeled with 6-FAM) depicting alleles of size 146 – 162bp. For a homozygous haplotype only one allele peak is seen (e.g. sample 01:37), for a heterozygous haplotype two allele peaks are graphed (e.g. sample 03:3).



4. RESULTS

4.1 Haplotype Distribution

With respect to all chromosomes under investigation, 6.6 alleles per locus were detected in the M1 line, with a range of 2 to 14 alleles per locus. On average, 8.2 alleles were detected for each locus of BTA 2 in the M1 line. The number of alleles ranged from 2 to 13. For BTA 6, the number of alleles per locus ranged from 3 to 10, with an average of 5.4 alleles per locus. On average, each locus of BTA 14 had 7.1 alleles detected. The number of alleles ranged from 2 to 12. Each locus of BTA 19 had, on average, 5.9 alleles per locus; ranging from 4 to 9 alleles per locus. The number of alleles per locus on BTA 21 ranged from 3 to 11, with an average of 6.5 alleles per locus. For each locus of BTA 23, 7.4 alleles were detected, on average. The number of alleles per locus ranged from 5 to 14. The frequencies of haplotypes of two adjacent loci that were analyzed for association ranged from 8.5% to 46.6% (Table 7.7).

4.2 Association between birth weight and common haplotypes

In total, 16 haplotypes were found to have significant association with birth weight (BWT) at the comparison wise threshold (Table 2). Among them, none reached the chromosome-wise threshold level. On BTA 2, two

haplotypes show significant association with birth weight. The two haplotypes spanned two different chromosomal regions. Haplotype TGLA 431-141/TEXAN 2-116 was located at 9.1cM to 22.5cM. It is associated with decreasing BWT by 0.36 S.D., or 1.61Kg. The haplotype TEXAN 5-147/BMS 356-99 is located at 95cM to 100.3cM. It was found to be associated with decreasing BWT by 0.32 S.D., or 1.43Kg.

On BTA 6, the chromosomal regions of 8.2cM to 11.8cM, 35.5cM to 49.7cM and 83cM to 86.2cM had five haplotypes associated with BWT, residing within them. The haplotypes were INRA 133-218/ILSTS 090-149 (located at 8.2cM-11.8cM), BM 1329-5/BMS 2508-102 (located at 35.5cM-44.2cM), BMS 2508-102/BMS 382-166 (located at 44.2cM-49.7cM), ILSTS 087-2/BM 1236-118 (located at 83cM-83.9cM) and BM 1236-122/BMS 2460-4 (located at 83.9cM-86.2cM). Haplotype INRA 133-218/ILSTS 090-149 was identified in the region of 8.2cM to 11.8cM. Haplotypes BM 1329-5/BMS 2508-102 and BMS 2508-102/BMS 382-166 were identified in the region of 35.5cM to 49.7cM. While haplotypes ILSTS 087-2/BM 1236-118 and BM 1236-122/BMS 2460-4, were identified in the region of 83cM to 86.2cM. The first four haplotypes showed 0.67, 0.69, 0.53 and 0.36 S.D. (or 3.00, 3.09, 2.37 and 1.36Kg) increases in BWT, respectively. While individuals with the last haplotype, BM 1236-122/BMS 2460-4, had a 0.55 S.D. (or 2.46Kg) decrease in BWT.

Three haplotypes occurring within three chromosomal regions on BTA 14, spanning 26cM to 29cM, 36.2cM to 46.2cM, and 52cM to 67.7cM were found to influence birth weight. The three haplotypes, BMS 1678-130/BMS 1941-111 (located in the region of 26cM-29cM), BMC 1207-151/BM 1577-152 (located in the region of 36.2cM-46.2cM) and BMS 1899-117/RM 137-100 (located at 52cM-67.7cM), are positively associated with birth weight. Respectively, they increase BWT by 0.56, 0.37 and 0.49 S.D. (or 2.51, 1.66 and 2.20Kg).

On BTA 19, animals with the haplotype BMS 2503-164/ BMS 2389-5 had 0.55 S.D. (or 2.46Kg) higher BWT than animals exhibiting other haplotypes. The haplotype resides in the area of 52cM to 52.7cM.

Three haplotypes, on BTA 21, including BMS 1117-95/AGLA 233-253 (located at 9.9cM to 20.4cM), BP 33-259/BMS 2815-95 (located at 28.2cM-46.1cM) and BP 33-271/BMS 2815-99, show significant positive effects on BWT of 0.65, 0.55 and 1.42 S.D. (or 2.91, 2.46 and 6.36Kg), respectively.

Two regions on BTA 23, located at 23.9cM to 36cM and 45.1cM to 50.9cM, were determined to have two haplotypes associated with birth weight residing within them. The haplotype BM 1258-103/BMS 468-125 (located at 23.9cM-36cM) had a 0.58 S.D. (or 2.60Kg) negative effect on BWT. While, haplotype RM 185-99/BM 1818-267 (residing at 45.1cM-50.9cM) had a positive effect on BWT of 0.50 S.D. (or 2.24Kg).

Table 2. Association between common haplotypes and BWT in the M1 line of *Bos taurus* from Beefbooster Inc. on BTA 2, 6, 14, 19, 21 and 23.

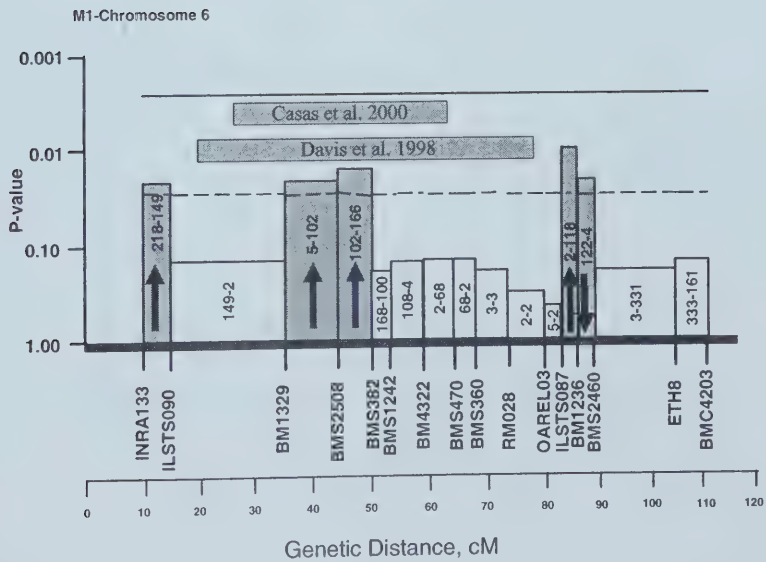
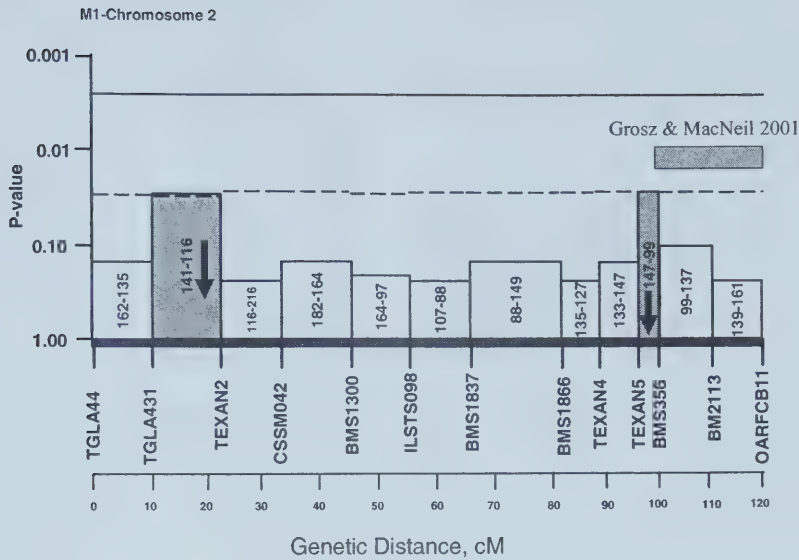
Haplotype ^A	Trait	BTA	P-value ^B	Haplotype effect ^C (Kg)
TGLA431-141, TEXAN2-116	BWT	2	0.0500*	-0.36 S.D (1.61)
TEXAN5-147, BMS356-99	BWT	2	0.0499*	-0.32 S.D (1.43)
INRA133-218, ILSTS090-149	BWT	6	0.0412*	+0.67 S.D (3.00)
BM1329-5, BMS2508-102	BWT	6	0.0394*	+0.69 S.D (3.09)
BMS2508-102, BMS382-166	BWT	6	0.0310*	+0.53 S.D (2.37)
ILSTS087-2, BM1236-118	BWT	6	0.0145*	+0.36 S.D (1.61)
BM1236-122, BMS2460-4	BWT	6	0.0247*	-0.55 S.D (2.46)
BMS1678-130, BMS1941-111	BWT	14	0.0059*	+0.56 S.D (2.51)
BMC1207-151, BM1577-152	BWT	14	0.0499*	+0.37 S.D (1.66)
BMS1899-117, RM137-100	BWT	14	0.0310*	+0.49 S.D (2.20)
BMS2503-164, BM2389-5	BWT	19	0.0346*	+0.55 S.D (2.46)
BMS1117-95, AGLA233-253	BWT	21	0.0187*	+0.65 S.D (2.91)
BP33-259, BMS2815-95	BWT	21	0.0443*	+0.55 S.D (2.46)
BP33-271, BMS2815-99	BWT	21	0.0065*	+1.42 S.D (6.36)
BM1258-103, BMS468-125	BWT	23	0.0211*	-0.58 S.D (2.60)
RM185-99, BM1818-267	BWT	23	0.0340*	+0.50 S.D (2.24)

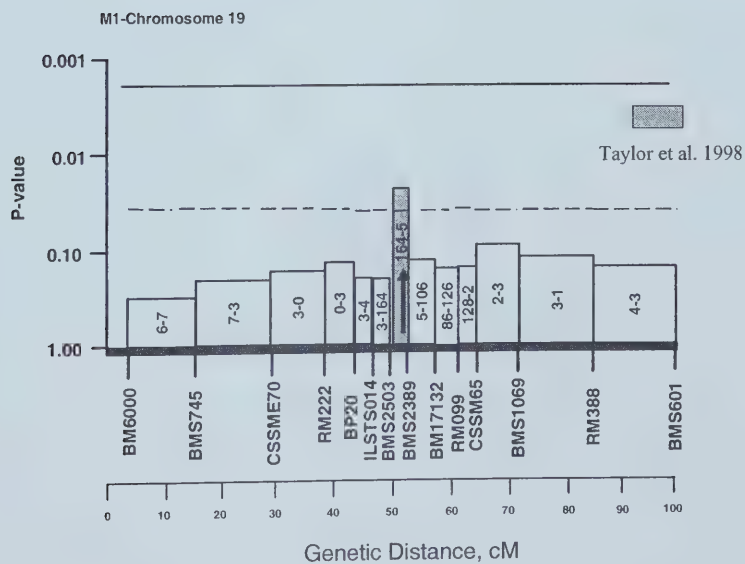
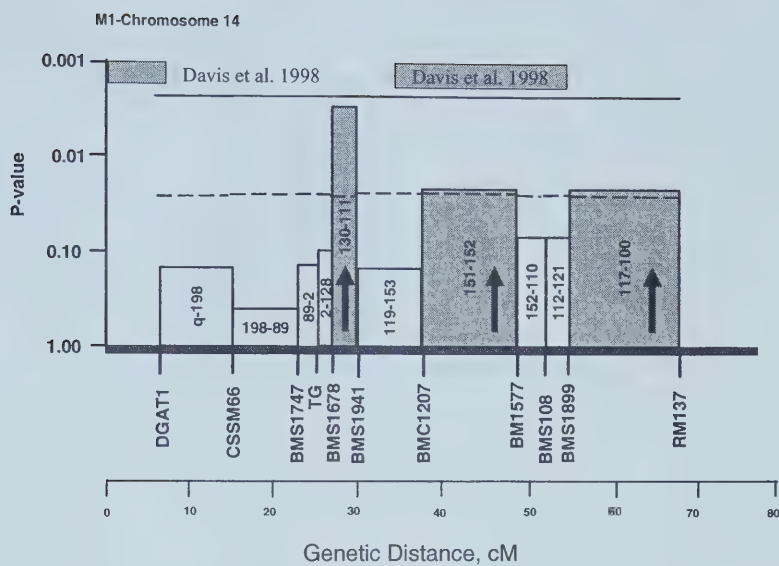
^AThe haplotypes were named by two alleles of a pair of loci. For example, haplotype RM185-99/BM1818-267 represented a segment of the chromosome having allele 99 of RM185 and allele 267 of BM1818.

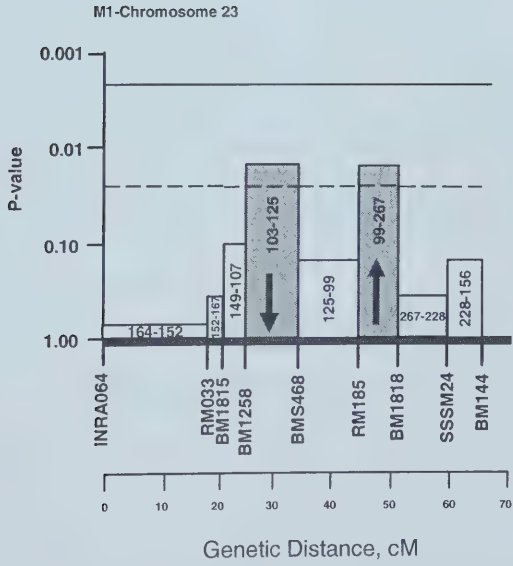
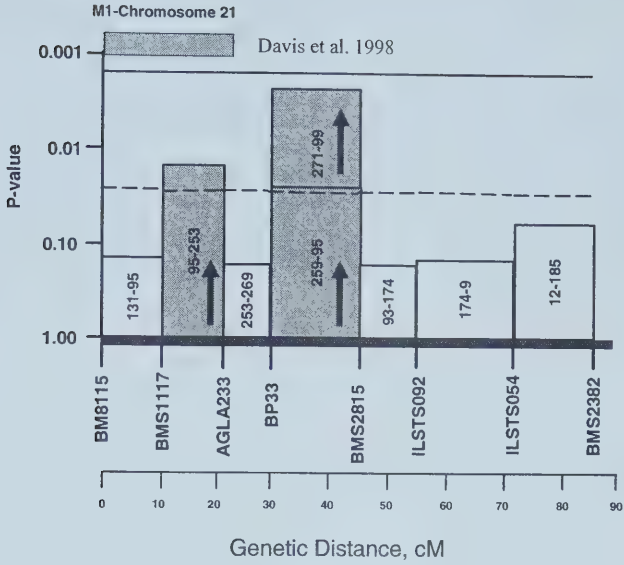
^B* indicated the P-values above the comparison-wise threshold level.

^CS.D. = standard deviation, “-” represented the negative effect, while “+” represented the positive effect. The value contained within () represents the effect in kilograms.

Figure 2 Haplotypes with lowest p-values between two adjacent loci along bovine chromosomes 2, 6, 14, 19, 21 and 23 for birth weight in the M1 commercial line of *Bos taurus* from Beefbooster Inc. Haplotypes were defined by two alleles of a pair of loci. For example, haplotype RM 185-99/BM 1818-267 represented a segment of chromosome having allele 99 of RM 185 and allele 267 of BM 1818. The genetic map distance was indicated in cM. The dashed line represented the comparison-wise threshold level, whereas the solid line represented the chromosome-wise p-value threshold level. Arrows indicate the effect of the haplotype (↑ indicates the haplotype had a positive effect on the growth trait, ↓ indicates the haplotype had a negative effect on the growth trait).







4.3 Association between pre-weaning average daily gain and common haplotypes

In total, 13 haplotypes were found to have significant association with pre-weaning average daily gain (PWADG), at the comparison-wise threshold level (Table 3). Among them, one reached the chromosome-wise threshold level. On BTA 2, none of the haplotypes tested showed association with pre-weaning average daily gain.

On BTA 6, the chromosomal regions of 11.8cM to 44.2cM and 83cM to 86.2cM had four haplotypes associated with PWADG residing within them. The haplotypes included ILSTS 090-149/BM 1329-5 (located at 11.8cM-35.5cM), BM 1329-5/BMS 2508-102 (located at 35.5cM-44.2cM), ILSTS 087-2/BM 1236-118 (located at 83cM-83.9cM) and BM 1236-118/BMS 2460-3 (located at 83.9cM-86.2cM). All four haplotypes had significant positive effects on PWADG of 0.46, 0.70, 0.37 and 0.52 S.D. (or 0.05, 0.07, 0.04 and 0.05Kg), respectively. The BM 1329-5/BMS 2508-102 (located at 35.5cM-44.2cM) haplotype showed significant association with PWADG at the chromosome-wise threshold level.

Three haplotypes occurring within one region on BTA 14, spanning 26.7cM to 50.8cM were found to influence PWADG. Individuals with the haplotypes: BMS 1941-119/BMC 1207-153 (located at 26.7cM-36.2cM), BMC 1207-153/BM 1577-152 (located at 36.2cM-46.2cM) and BM 1577-152/BMS 108-

110 (located at 46.2cM-50.8cM) were found to show a positive effect of 0.67, 0.42 and 0.68 S.D. (or 0.07, 0.04 and 0.07Kg), respectively, on PWADG.

On BTA 19, one haplotype, BM 6000-6/BMS 745-7 showed association with PWADG. The haplotype was localized to the region between 4.8cM and 15.9cM. Individuals with the haplotype were found to have a 0.45 S.D. (or 0.05Kg) decrease in PWADG versus individuals with other haplotypes.

On BTA 21, one haplotype was found to have significant association with PWADG. Haplotype BMS 1117-95/AGLA 233-253 (located at 9.9cM to 20.4cM) had a 0.70 S.D. (or 0.07Kg) positive effect on PWADG.

On BTA 23, four haplotypes, situated within two regions (17.3cM to 36cM and 45.1cM to 50.9cM), were associated with PWADG. The haplotypes RM 033-152/BM 1815-151 (located at 17.3cM-19.8cM), BM 1815-149/BM 1258-107 (located at 19.8cM-23.9cM) and BM 1258-103/BMS 468-127 (located at 23.9cM-36cM) had negative effects of 0.72, 0.57 and 0.80 S.D. (or 0.07, 0.06 and 0.08Kg), respectively. All three haplotypes reside within the 17.3cM to 36cM area. Animals with the haplotype RM 185-99/BM 1818-267, located at 45.1cM to 50.9cM, showed a 0.40 S.D. (or 0.04Kg) increase in PWADG over animals with other haplotypes.

Table 3 Association between haplotypes and PWADG in the M1 line of *Bos taurus* from Beefbooster Inc on BTA 2, 6, 14, 19, 21 and 23.

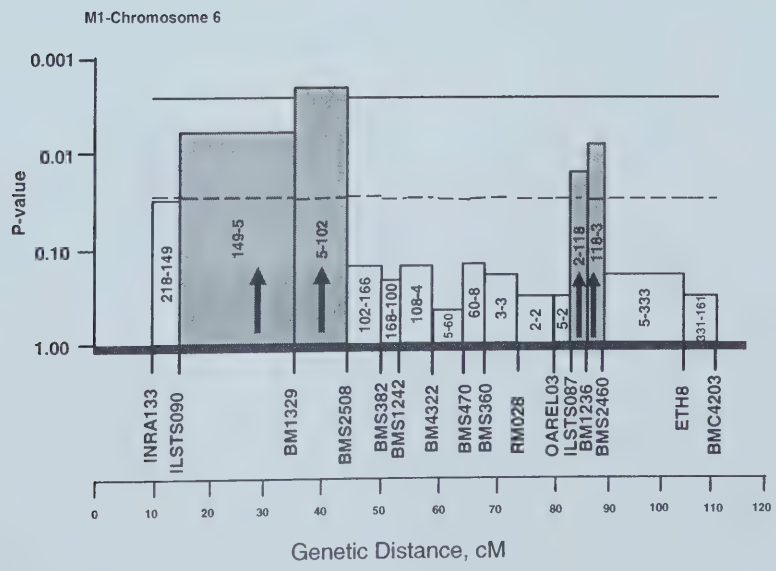
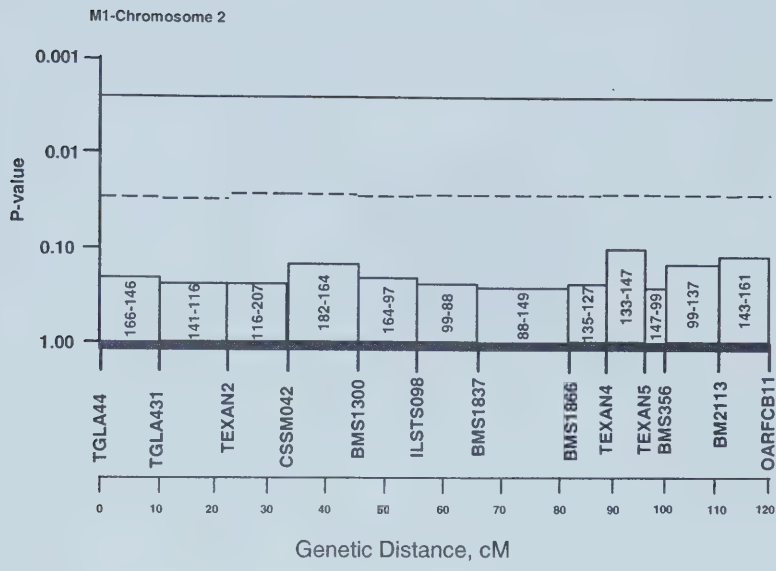
Haplotype ^A	Trait	BTA	P-value ^B	Haplotype effect ^C (Kg)
ILSTS090-149, BM1329-5	PWADG	6	0.0089*	+0.46 S.D (0.05)
BM1329-5, BMS2508-102	PWADG	6	0.0037**	+0.70 S.D (0.07)
ILSTS087-2, BM1236-118	PWADG	6	0.0337*	+0.37 S.D (0.04)
BM1236-118, BMS2460-3	PWADG	6	0.0196*	+0.52 S.D (0.05)
BMS1941-119, BMC1207-153	PWADG	14	0.0457*	+0.67 S.D (0.07)
BMC1207-153, BM1577-152	PWADG	14	0.0227*	+0.42 S.D (0.04)
BM1577-152, BMS108-110	PWADG	14	0.0411*	+0.68 S.D (0.07)
BM6000-6, BMS745-7	PWADG	19	0.0495*	-0.45 S.D (0.05)
BMS1117-95, AGLA233-253	PWADG	21	0.0451*	+0.70 S.D (0.07)
RM033-152, BM1815-151	PWADG	23	0.0378*	-0.72 S.D (0.07)
BM1815-149, BM1258-107	PWADG	23	0.0135*	-0.57 S.D (0.06)
BM1258-103, BMS468-127	PWADG	23	0.0064*	-0.80 S.D (0.08)
RM185-99, BM1818-267	PWADG	23	0.0496*	+0.40 S.D (0.04)

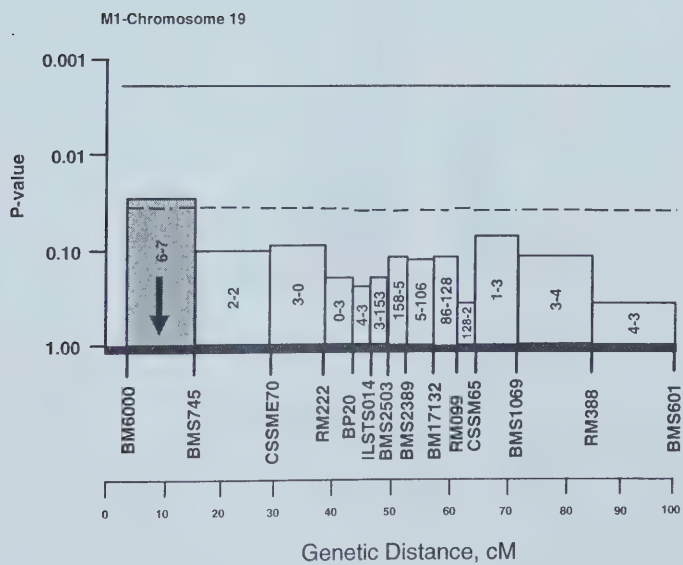
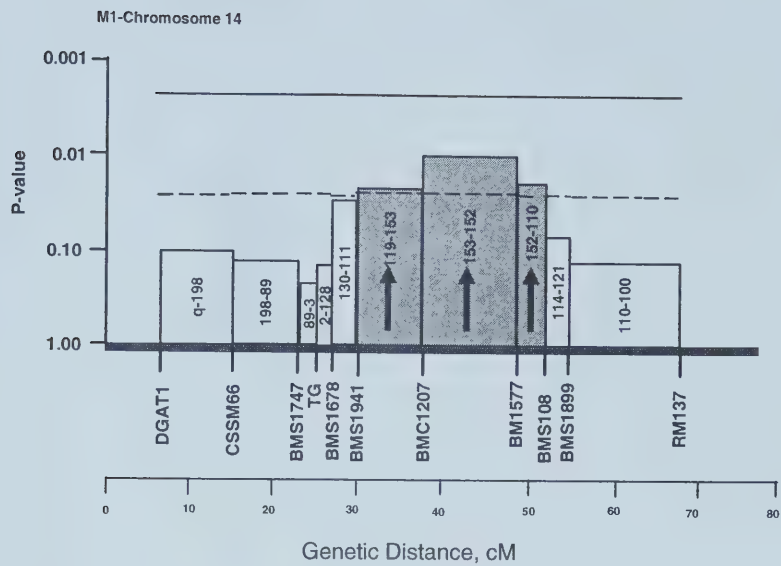
^AThe haplotypes were named by two alleles of a pair of loci. For example, haplotype RM185-99/BM1818-267 represented a segment of the chromosome having allele 99 of RM185 and allele 267 of BM1818.

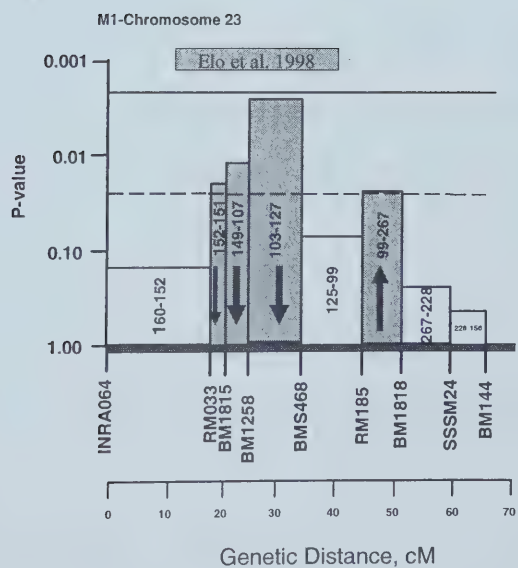
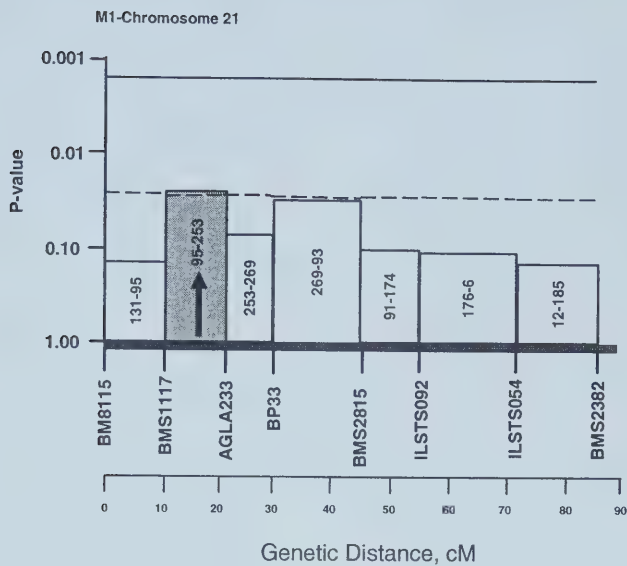
^B* and ** indicated the P-values above the comparison-wise and the chromosome-wise thresholds, respectively.

^CS.D. = standard deviation, “-” represented the negative effect, while “+” represented the positive effect. The value contained within () represents the effect in kilograms.

Figure 3 Haplotypes with lowest p-values between two adjacent loci along bovine chromosomes 2, 6, 14, 19, 21 and 23 for pre-weaning average daily gain in the M1 commercial line of *Bos taurus* from Beefbooster Inc. Haplotypes were defined by two alleles of a pair of loci. For example, haplotype RM 185-99/BM 1818-267 represented a segment of chromosome having allele 99 of RM 185 and allele 267 of BM 1818. The genetic map distance was indicated in cM. The dashed line represented the comparison-wise threshold level, whereas the solid line represented the chromosome-wise p-value threshold level. Arrows indicate the effect of the haplotype (↑ indicates the haplotype had a positive effect on the growth trait, ↓ indicates the haplotype had a negative effect on the growth trait).







4.4 Association between post-weaning average daily gain on feed and common haplotypes

In total, 11 haplotypes were found to have significant association with post-weaning average daily gain on feed (ADGF), at the comparison-wise threshold level (Table 4). Among them, two reached the chromosome-wise threshold level. On BTA 2, the haplotype TGLA 431-141/TEXAN 2-116 (located in the area of 9.1cM to 22.5cM) was associated with ADGF. Animals with the haplotype exhibited 0.39 S.D. (or 0.07Kg) higher ADGF than animals with other haplotypes.

On BTA 6, the chromosomal regions of 49.7cM to 50.1cM and 59.6cM to 63.6cM had three haplotypes with significant effects on ADGF residing within them. Haplotype BMS 382-168/BMS 1242-100 (located at 49.7cM-50.1cM) had a negative effect of 0.70 S.D. (or 0.13Kg). A negative effect of 0.99 S.D. (or 0.18Kg) was noted in individuals with the haplotype BMS 4322-5/BMS 470-60. Individuals with the haplotype BM 4322-2/BMS 470-68, located in the region of 59.6cM to 63.6cM, showed an increase in ADGF of 1.37 S.D. (or 0.25Kg).

Three haplotypes occurring within two chromosomal regions on BTA 14, spanning 17cM to 25.5cM and 36.2cM to 46.2cM were found to influence post-weaning average daily gain on feed (ADGF). Animals with the haplotype CSSM 66-198/BMS 1747-89 (located at 17cM-22cM) or the haplotype BMS 1747-89/TG-2 (located at 22cM-24cM) showed 0.89 S.D. and

0.50 S.D. (or 0.16 and 0.09Kg) greater ADGF, respectively. Meanwhile, individuals with the haplotype BMC 1207-153/BM 1577 (located in the region of 36.2cM to 46.2cM), showed a decrease in ADGF of 0.73 S.D. (or 0.15Kg).

On BTA 19, two QTL regions located at 52cM to 52.7cM and 65.1cM to 65.7cM were identified. Individuals with BMS 2503-158/BMS 2389-2 or BMS 2503-164/BMS 2389-5, both in the area of 52cM to 52.7cM, for a haplotype showed higher ADGF of 0.91 and 0.86 S.D. (or 0.16 and 0.15Kg), respectively; whereas those with the haplotype RM 099-128/CSSM 65-2, in the area of 65.1cM to 65.7cM, were found to have a 0.54 S.D. (or 0.10Kg) lower ADGF. The haplotypes BMS 2503-158/BMS 2389-2 and BMS 2503-164/BMS 2389-5 were determined to have significant association with ADGF at the chromosome-wise threshold level.

On BTA 21, animals with the haplotype BMS 2815-93/ILSTS 092-174 exhibited 0.88 S.D. higher ADGF over animals with other haplotypes. The haplotype falls within the region of 46.1cM to 53.1cM.

Of the haplotypes analyzed on BTA 23, none were found to have significant effects on ADGF.

Table 4 Association between haplotypes and ADGF in the M1 of *Bos taurus* from Beefbooster Inc on BTA 2, 6, 14, 19, 21 and 23.

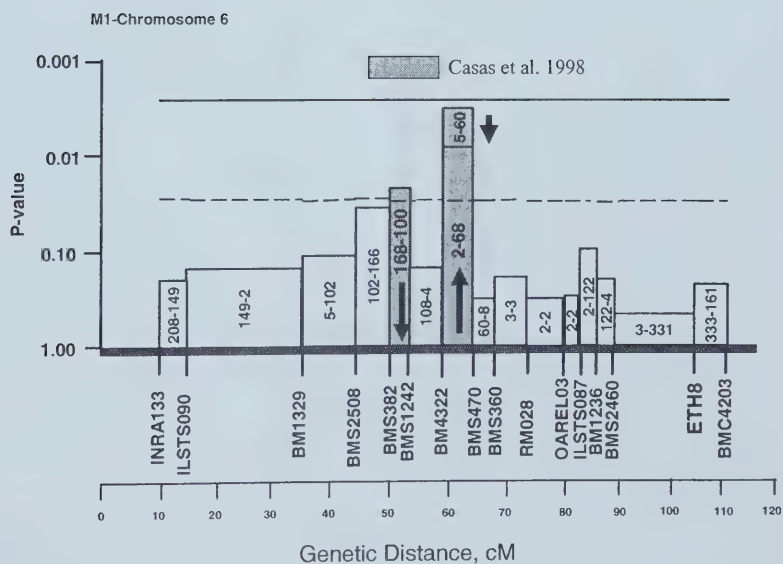
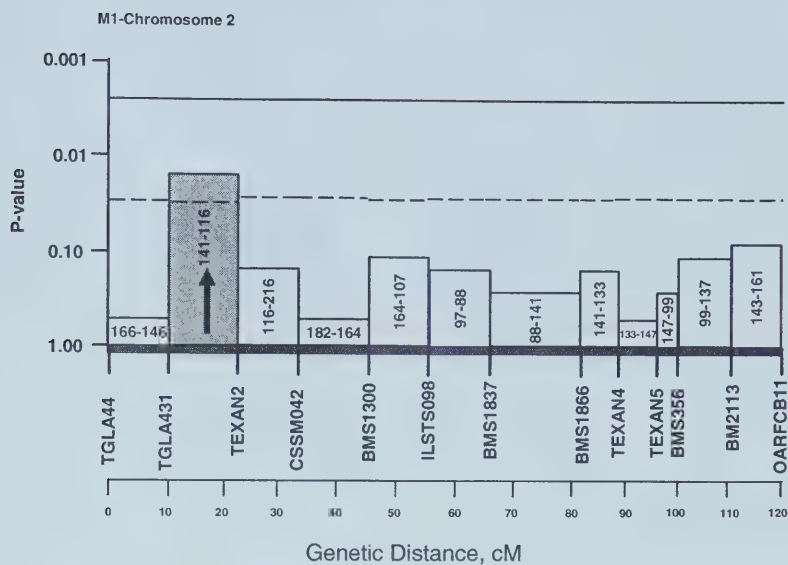
Haplotype ^A	Trait	BTA	P-value ^B	Haplotype effect ^C (Kg)
TGLA431-141, TEXAN2-116	ADGF	2	0.0321*	+0.39 S.D (0.07)
BMS382-168, BMS1242-100	ADGF	6	0.0340*	-0.70 S.D (0.13)
BM4322-5, BMS470-60	ADGF	6	0.0043*	-0.99 S.D (0.18)
BM4322-2, BMS470-68	ADGF	6	0.0080*	+1.37 S.D (0.25)
CSSM66-198, BMS1747-89	ADGF	14	0.0248*	+0.89 S.D (0.16)
BMS1747-89, TG-2	ADGF	14	0.0261*	+0.50 S.D (0.09)
BMC1207-153, BM1577-143	ADGD	14	0.0389*	-0.73 S.D (0.15)
BMS2503-158, BM2389-2	ADGF	19	0.0039**	+0.91 S.D (0.16)
BMS2503-164, BM2389-5	ADGF	19	0.0010**	+0.86 S.D (0.15)
RM099-128, CSSM65-2	ADGF	19	0.0140*	-0.54 S.D (0.10)
BMS2815-93, ILSTS092-174	ADGF	21	0.0126*	+0.88 S.D (0.16)

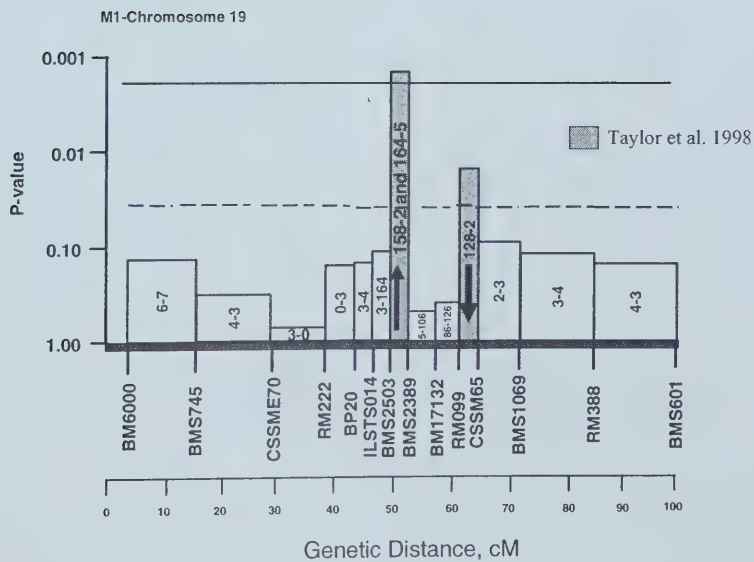
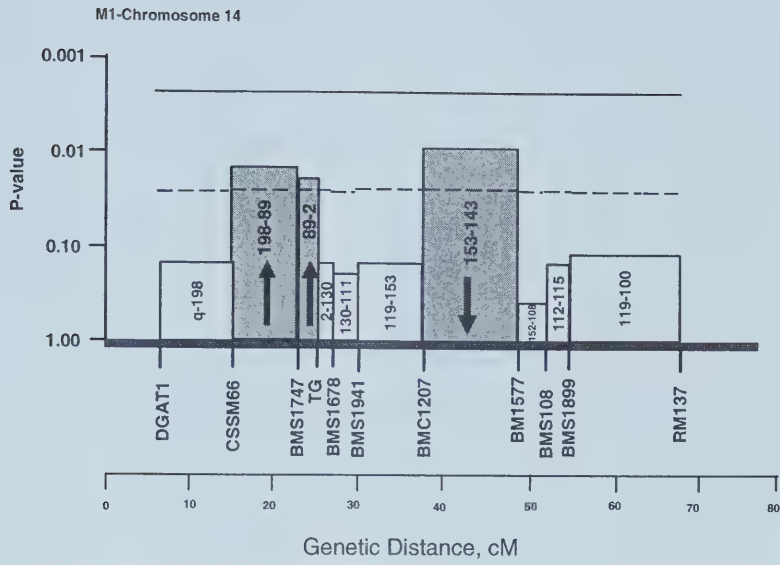
^AThe haplotypes were named by two alleles of a pair of loci. For example, haplotype RM099-128/CSSM65-2 represented a segment of the chromosome having allele 128 of RM099 and allele 2 of CSSM65.

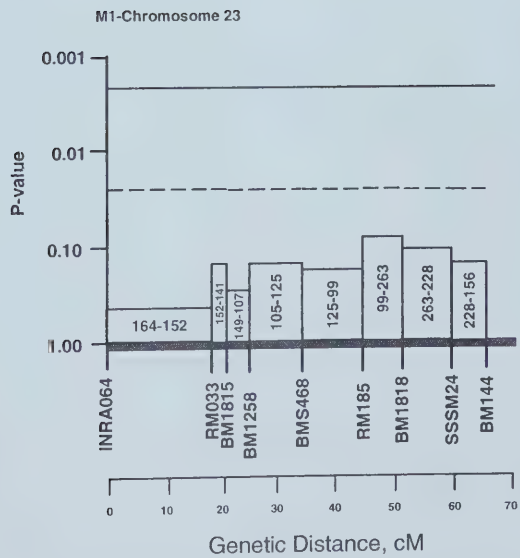
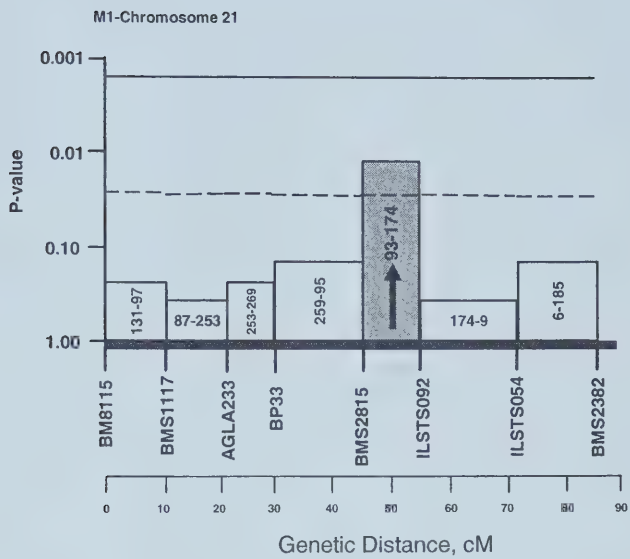
^B* and ** indicated the P-values above the comparison-wise and the chromosome-wise thresholds, respectively.

^cS.D. = standard deviation, “-” represented the negative effect, while “+” represented the positive effect. The value contained within () represents the effect in kilograms.

Figure 4 Haplotypes with lowest p-values between two adjacent loci along bovine chromosomes 2, 6, 14, 19, 21 and 23 for post-weaning average daily gain on feed in the M1 commercial line of *Bos taurus* from Beefbooster Inc. Haplotypes were defined by two alleles of a pair of loci. For example, haplotype RM 185-99/BM 1818-267 represented a segment of chromosome having allele 99 of RM 185 and allele 267 of BM 1818. The genetic map distance was indicated in cM. The dashed line represented the comparison-wise threshold level, whereas the solid line represented the chromosome-wise p-value threshold level. Arrows indicate the effect of the haplotype (↑ indicates the haplotype had a positive effect on the growth trait, ↓ indicates the haplotype had a negative effect on the growth trait).







5. DISCUSSION

5.1 Birth weight

Birth weight is an important economic trait in the beef cattle industry. Dams producing calves with a birth weight that is either too low, or too high, are typically culled from the herd. Calves with low birth weight are more highly subjective to morbidity and mortality, they tend to be smaller at weaning, they are not as feed efficient, nor cost efficient. Calves with high birth weight often cause dystocia or other calving difficulties to occur, they result in delayed rebreeding and reduced conception of the dam, there is an increased risk of morbidity and mortality of both the calf and the dam, and the calf is not as feed efficient, or as cost efficient. Therefore, selection is applied for the production of calves with a birth weight that is neither too low, nor too high, but is usually higher than lower to ensure more efficient rate of gain.

In this study, QTL for birth weight were identified on BTA 2 in animals with the haplotype TGLA 431-141/TEXAN 2-116 (9.1cM to 22.5cM) or the haplotype TEXAN 5-147/BMS 356-99 (95cM to 100.3cM). Both haplotypes had a negative effect on birth weight, i.e. birth weight was lower in individuals possessing either haplotype. Quantitative trait loci for birth weight have been previously mapped to BTA 2 in the region of 108cM to 122cM in the interval between BM 2113 and OarFCB 11, with maximum

effect at 114cM (Grosz & MacNeil 2001); this is similar to the region identified herein.

Grosz and MacNeil (2001) focused on a whole genome scan using the progeny of a single Hereford X composite bull and 170 microsatellites from the 29 autosomes. The composite breed consisted of $\frac{1}{2}$ Angus, $\frac{1}{4}$ Tarentaise and $\frac{1}{4}$ Charolais. Interestingly, the Beefbooster M1 line was also developed from an Angus base, thus common ancestry is possible. Microsatellite markers used by Grosz and MacNeil (2001) that were heterozygous were spaced at approximately 20cM intervals throughout the genome. The genetic markers used here were not required to be heterozygous and were spaced at closer intervals (<15cM). These differences could account for the slight discrepancy in the position of the mapped QTL. Grosz and MacNeil (2001) suggest that previous experiments used more diverse populations and thus QTL detection was more likely in those studies. The animals used in the current study would likely be more diverse than those used by Grosz and MacNeil (2001); this could also be a reason for the slight difference in mapped QTL position.

Quantitative trait loci for BWT were identified on BTA 6 in three chromosomal regions including 8.2cM to 11.8cM (haplotype INRA 133-218/ILSTS 090-149), 44.2 cM to 50.1cM (haplotypes: BM 1329-5/BMS 2508-102 and BMS 2508-102/BMS 382-166) and 83cM to 83.9cM (haplotypes: ILSTS 087-2/BM 1236-118 and BM 1236-122/BMS 2460-4).

With the exception of haplotype BM 1236-122/BMS 2460-4, which had a negative effect on birth weight, all other haplotypes were shown to have a positive effect on birth weight. Other studies have also identified QTL for birth weight on BTA 6. Davis and colleagues (1998) report finding a QTL for BWT in the chromosomal region of 15cM to 75cM, with the highest peak around 48cM. This group used a herd of tropical beef cattle and genetic markers were chosen from the map produced on the International Bovine Reference Panel (IBRP). The peak at 48cM lies within the region encompassed by the haplotypes BM 1329-5/BMS 2508-102 and BMS 2508-102/BMS 382-166, situated at 44.2cM to 50.1cM, reported in this study.

Casas and associates (2000) identified a QTL for BWT on BTA 6 in the region of 25cM to 60cM, peaking around 45cM to 55cM. In animals inheriting the MARC III allele higher birth weights were noted. The chromosomal region identified is in agreement with what was found in the current study. It is important to note that the MARC III is a composite breed comprised of $\frac{1}{4}$ Angus, $\frac{1}{4}$ Hereford, $\frac{1}{4}$ Red Poll and $\frac{1}{4}$ Pinzgauer. Although some of the breeds are common between the Casas et al. study and the Beefbooster breeding lines, there are many populations of the different breeds all over the world, thus the QTL region similarity could be coincidental.

Davis and associates (1998) have mapped QTLs for BWT in the regions of both 0cM to 5cM and 35cM to 55cM on BTA 14. The current study also identified QTLs for BWT on BTA 14. They were determined to be located at

26cM to 26.7cM (haplotype BMS 1678-130/BMS 1941-111), 36.2 cM to 46.2cM (haplotype BMC 1207-151/BM 1577-152) and 52 cM to 67.7cM (haplotype BMS 1899-117/RM 137-100). These haplotypes were determined to each have a positive effect on birth weight. Only one of these regions, 36.2cM to 46.2cM, with haplotype BMC 1207-151/BM 1577-152, is in agreement with Davis et al. (1998). Davis and associates (1998) utilized a tropical beef cattle herd, using breeds different from the Beefbooster line. The other chromosomal regions identified in the current study as housing QTLs could signify new QTL, or QTL that are segregating within the lines studied here, but not in the tropical beef herd used in the Davis et al. (1998) study.

Bovine chromosome 19 was a likely chromosome to target because the growth hormone 1 gene (GH1) had been previously mapped to it. In a study evaluating the support for candidate genes as QTLs, on BTA 19, (Taylor et al. 1998) it was reported that a QTL for birth weight, in the region of 95cM to 105cM, may exist, though the support was weak. The current study identified a possible QTL for BWT, at the comparison-wise threshold level. Animals with the haplotype BMS 2503-164/BMS 2389-5, located in the chromosomal region of 52cM to 52.7cM, showed an increase in birth weight over animals exhibiting other haplotypes. This region has not been reported in other studies, whether it is a new QTL region is yet to be determined. For new regions identified in this study, other studies must be performed in order to confirm the regions identified here.

Two regions on BTA 21 (9.9cM to 20.4cM and 28.2cM to 46.1cM) were found to have QTLs for birth weight. Three haplotypes, responsible for higher BWT at the comparison-wise threshold level, have been identified. They are, BMS 1117-95/AGLA 233-253 (located at 9.9cM to 20.4cM), BP 33-259/BMS 2815-95 (located at 28.2cM to 46.1cM) and BP 33-271/BMS 2815-99 (located at 28.2cM to 46.1cM). All three haplotypes were shown to have significant positive effects on BWT. Previously, a QTL for birth weight was mapped in the area surrounding 0cM to 4cM on BTA 21 (Davis et al. 1998). This is in close proximity to one of the regions identified here, 9.9cM to 20.4cM. As noted previously, the herd utilized in the Davis et al. (1998) study was a tropical beef herd. Whether the QTL identified in this region is the same as that found by Davis et al. (1998), or is a new QTL is not clear at the present.

We have identified two regions in which birth weight QTL may exist on BTA 23. These two regions include 23.9cM to 36cM and 45.1cM to 50.9cM. Two haplotypes (BM 1258-103/BMS 468-125 and BM 1258-103/BMS 468-127 both located at 23.9cM to 36cM) had negative impact on birth weight, while the haplotype RM 185-99/BM 1818-267 (in the region of 45.1cM to 50.9cM) had a positive effect on birth weight at the comparison-wise threshold level. To date, no other studies confirm QTL for birth weight on BTA 23.

5.2 Pre-weaning average daily gain

Pre-weaning gain is an important economic trait, as many beef producers will market their calves soon after weaning. Therefore, it is the weight they have gained prior to this, that is of interest to the cattle producer. There were no QTL regions affecting PWADG identified on BTA 2 in the current study. At the present time no other studies report QTLs for this trait on BTA 2.

Two haplotypes were identified on BTA 6 in the region of 11.8cM to 44.2cM (ILSTS 090-149/BM 1329-5 and BM 1329-5/BMS 2508-102) that have a significant effect on increasing PWADG. The haplotype BM 1329-5/BMS 2508-102 is significant at the chromosome-wise threshold level. We have also found two haplotypes in the region of 83cM to 86.2cM (ILSTS 087-2/BM 1236-118 and BM 1236-118/BMS 2460-3) that have a positive effect on PWADG. No other studies at this time report QTLs for weaning weight or pre-weaning average daily gain in these regions.

Previous studies reported QTL for carcass characteristics such as longissimus muscle area, fat thickness and fat depth on BTA 14 (Stone et al. 1999, Casas et al. 2000). However, no other studies were found to identify QTL for pre-weaning gain on BTA 14. We report QTL for PWADG in the region of 26.7cM to 50.8cM. Animals with any one of the three haplotypes: BMS 1941-119/BMC 1207-153, BMC 1207-153/BM 1577-152 or BM 1577-152/BMS 108-110 showed significant increases in PWADG, at the comparison-wise threshold level.

On BTA 19, a QTL for lower PWADG was identified in the area of 4.8cM to 15.9cM in individuals with the haplotype BM 6000-6/BMS 745-7. Individuals with this haplotype have a lower PWADG. Although previous studies focused on BTA 19 because it houses the GH1 gene and reportedly houses QTLs for birth weight and post-weaning average daily gain, no information is available suggesting other QTLs have been found that influence pre-weaning average daily gain. Whether the QTL identified here for PWADG is a new QTL is unclear at the present time.

On BTA 21, haplotype BMS 1117-95/AGLA 233-253 (located at 9.9cM to 20.4cM) was found to have an association with increased PWADG at the comparison-wise threshold. At the present time, no other studies have identified QTL for weaning weight or pre-weaning average daily gain on BTA 21.

Elo and associates (1999) were searching for QTLs affecting quantitative traits in dairy cattle including live weight, which was subsequently mapped to BTA 23. The QTL was mapped to a region spanning 12cM to 41cM, with a peak at 22cM to 35cM. The mapping of a QTL for live weight on BTA 23 was the primary reasoning behind choosing to fine map this chromosome. In the area of 17.2cM to 23.9cM two haplotypes were found to reduce PWADG. One of the regions we have identified as possibly housing QTLs for growth traits (17.2cM to 36cM) is in agreement with Elo et al. (1999); the other region we identified is close to that established by Elo and associates. In

addition, two other regions in which QTL may exist have been identified in the current study. These two regions include 17.2cM to 36cM and 45.1cM to 50.9cM. Haplotype BM 1258-103/BMS 468-127 (located at 23.9cM to 36cM) had a negative impact on pre-weaning average daily gain, while the haplotype RM 185-99/BM 1818-267 (spanning 45.1cM to 50.9cM) had a positive effect on pre-weaning average daily gain at the comparison-wise threshold level. Selection for lower birth weight often results in the lowering of pre- and post-weaning gain, subsequently selecting for higher pre- or post-weaning gain often results in higher birth weights. With respect to BTA 23, we found that haplotypes were associated with a reduction of either PWADG or ADGF. It appears that although selection for birth weight fell within the limits, as outlined by MacNeil and Newman (1994), this led to a reduction in pre-weaning gain, although obviously not enough to justify culling the individuals segregating the haplotypes associated with this decrease.

5.3 Post-weaning average daily gain on feed

In the current study, a QTL for post-weaning average daily gain on feed at 9.1cM to 22.5cM in individuals possessing the TGLA 431-141, TEXAN 2-116 haplotype on BTA 2 was found. This particular haplotype is associated with an increase in ADGF. This is the same haplotype identified as lowering birth weight, in this study. This result would be highly sought by cattle producers. Lower birth weight would decrease the likelihood of dystocia, and

higher post-weaning gain ultimately means higher economic returns for producers. Typically, calves with lower birth weight would also have lower pre-weaning average daily gain and lower post-weaning average daily gain on feed. As stated previously, Grosz & MacNeil (2001) found a QTL associated with birth weight on BTA 2 in the interval encompassing BM 2113 and OarFCB 11; this QTL was responsible for a decrease in birth weight. It is interesting to note that Grosz & MacNeil (2001) found no significant effects on pre- or post-weaning gain in the calves with lower birth weight.

In Belgian Blue and Piedmontese cattle, the *mh* gene associated with double muscling, was thought to be located near the COL3A1 gene (Charlier et al. 1995, Casas et al. 1997, Sonstegard et al. 1997) which happens to be in close proximity to microsatellite TGLA 431, a microsatellite used in the current study. In cattle possessing the double muscling phenotype, there is an increased proficiency in converting feed to muscle. Myostatin (or GDF-8), a gene causing muscular hypertrophy in mice (McPherron et al. 1997), was found to be located in the interval in which bovine *mh* was mapped (Kambadur et al. 1997). Bovine myostatin has since been localized to a position between microsatellite TGLA 44 and the COL3A1 gene; it is located 1.9cM from TGLA 44 and 2.0cM from COL3A1 (Smith et al. 1997b).

Studies show that, DM/DM (homozygous for double muscling) calves tend to have higher birth weights and greater birth to weaning gain than N/N (two normal alleles at the double muscling locus) calves. However, post-weaning

growth rate of DM/DM calves is less than that of N/N calves such that at slaughter there is little to no difference in live weights between the groups; while heterozygous DM/N calves tend to display higher weaning and yearling weights than calves from either of the homozygous groups (Casas et al. 1999). These observations could be useful in explaining the higher ADGF noted here. Although double muscling has been so strongly selected for, such that it has been fixed in certain breeds, perhaps a QTL linked to the *mh* locus, or found in the same area, has been segregating in other cattle populations as well and is responsible for influencing post-weaning gain.

Some researchers suggest that Asturiana, Chianina, Charolais, Angus, and Limousin breeds may also express double muscling (Grobet et al. 1997, McPherron & Lee 1997). It is important to note that the Beefbooster M1 line was developed from an Angus base. In addition, great emphasis was placed in selecting for high weaning weight in the M1 line. Selection for high weaning weights, but culling individuals with too high a birth weight seems to have resulted in the segregation of haplotypes for both low birth weight and higher post-weaning gain in the population.

On BTA 6, three haplotypes were found to have significant effects on ADGF. Individuals possessing the haplotypes BMS 382-168/BMS 1242-100 or BM 4322-5/BM 4322-60 (located in the regions of 49.7cM to 50.1cM and 59.6cM to 63.6cM, respectively) displayed lower ADGF, while individuals with the haplotype BM 4322-2/BMS 470-68 (located in the area of 59.6cM to 63.6cM)

showed an increase in ADGF. Casas and associates (2000) report finding a QTL for yearling weight around 48cM-58cM on BTA 6. Animals inheriting the MARC III allele, which includes Angus in its background, had higher yearling weight than animals that did not inherit the MARC III allele. The potential QTL found in the Casas et al. (2000) study is in close proximity to both BMS 382-168/BMS 1242 and BM 4322-2/BMS 470-68. There are slight differences in QTL location and effect reported here as compared to the Casas et al. (2000) study, whether there are one or more new QTLs present in the locations specified is unclear at the present. The study by Casas and associates (2000) mapped QTL for growth and carcass composition in cattle segregating alternate forms of myostatin, which could be responsible for the subtle differences noted here.

A QTL for ADGF was identified at 16.5cM to 25.5cM on BTA 14 has been identified here. Two haplotypes: CSSM 66-198/BMS 174-89 and BMS 1747-89/TG-2, reside within this region and are responsible for increasing ADGF. In addition, a QTL was also located in the area of 36.2cM to 46.2cM in individuals possessing the haplotype BMC 1207-153/BM 1577-143. The haplotype was responsible for lowering ADGF. No other studies were found to identify QTL for post-weaning gain on BTA 14.

In a study on BTA 19 by Taylor et al. (1998) it was reported that a QTL for post-weaning average daily gain on feed in the region of 80cM to 85cM may exist, though the support was weak. In the current study a suggestive QTL for

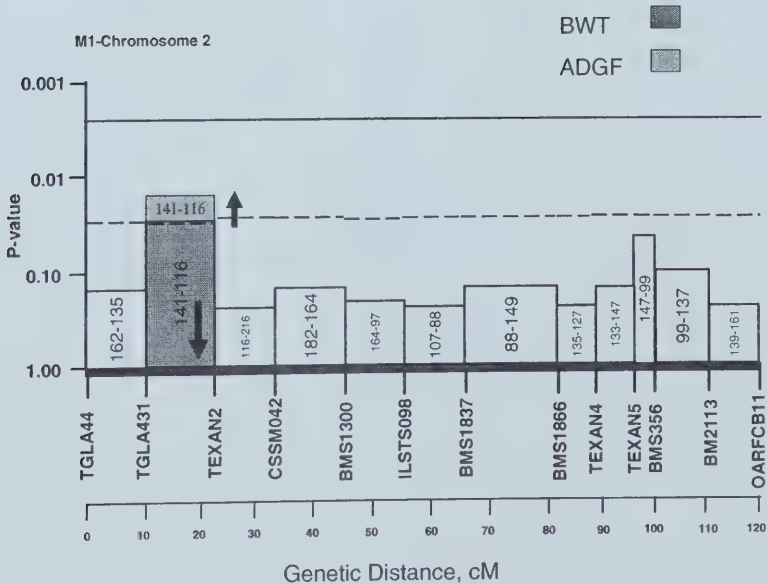
ADGF was also identified, however it resides at 65.1cM to 65.7cM in individuals possessing the RM 099-128, CSSM 65-2 haplotype. Individuals with the haplotype have higher ADGF. In addition, another QTL for ADGF, at the chromosome-wise threshold, in animals with the haplotype BMS 2503-164/BMS 2389-5 or the haplotype BMS 2503-158/BMS 2389-2 located at 52cM to 52.7cM was also identified. Both haplotypes have positive effects on ADGF.

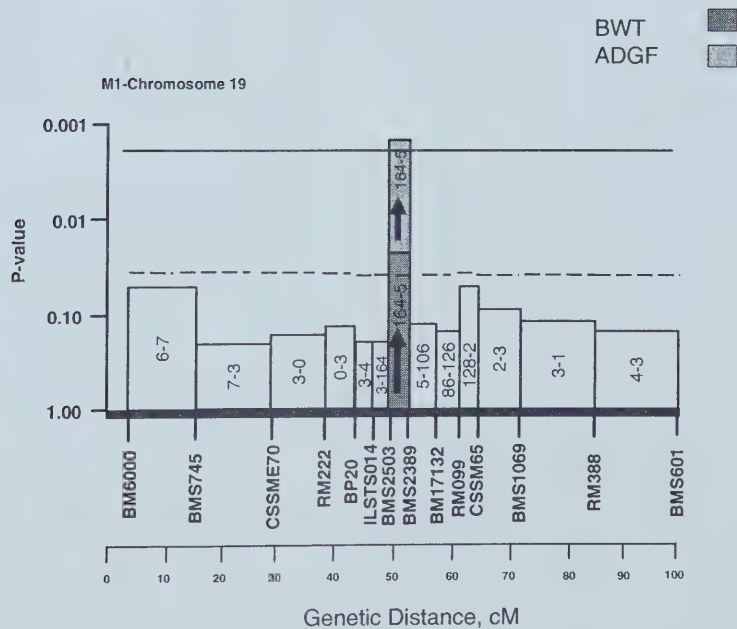
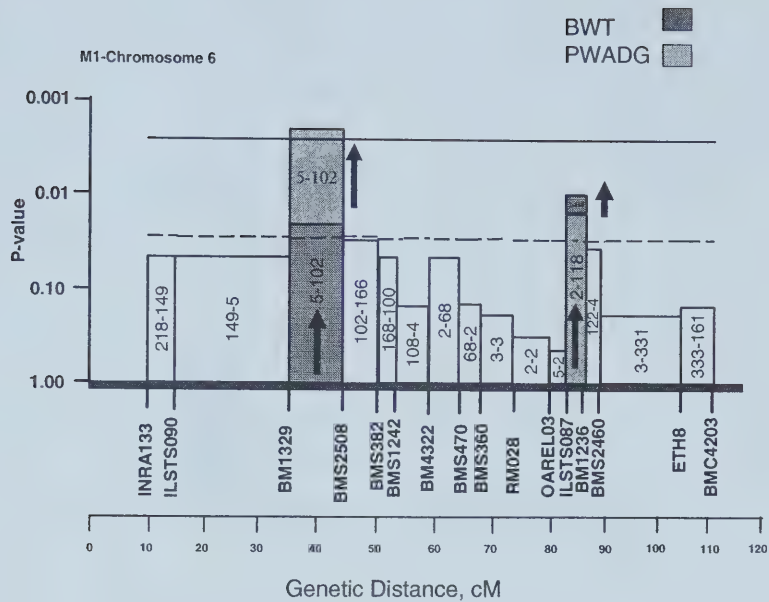
On BTA 21, a QTL for ADGF was found at 46.1cM to 53.1cM in individuals with the haplotype BMS 2815-93/ILSTS 092-174. Animals with the haplotype showed increased ADGF over animals with other haplotypes. At the present, no other studies report QTL for ADGF on BTA 21.

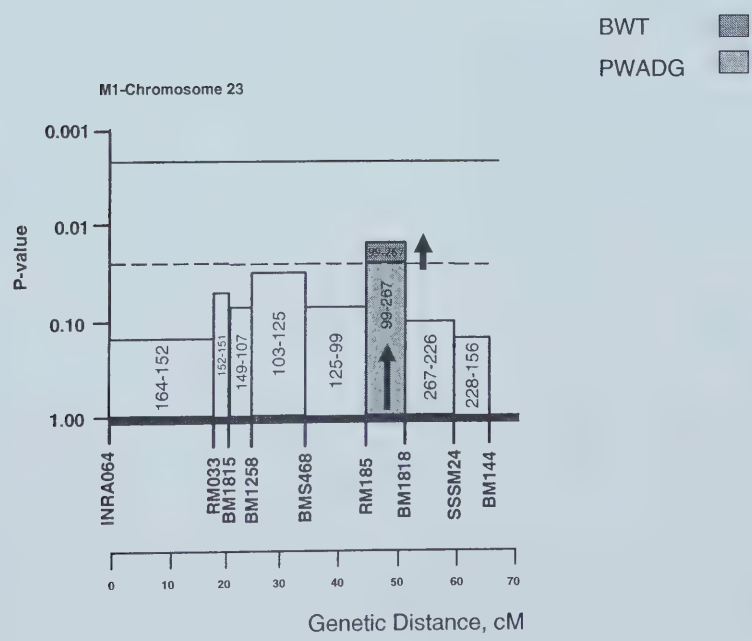
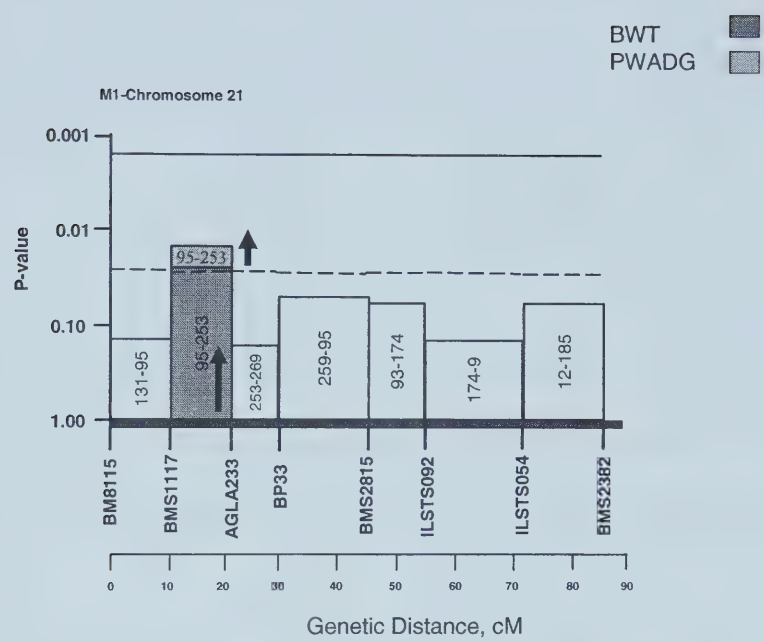
Some haplotypes were associated with more than one growth trait (Figure 5). On BTA 2 the haplotype TGLA 431-141/TEXAN 2-116 was associated with lower BWT and higher ADGF. On BTA 6, two haplotypes were associated with an increase in BWT and a concomitant increase in PWADG. On BTA 19 a haplotype associated with higher BWT was also associated with higher ADGF. On BTA 21 one haplotype associated with higher BWT was also associated with a subsequently higher PWADG. Finally, on BTA 23 a haplotype associated with higher BWT was also associated with higher PWADG.

Figure 5

Haplotypes with lowest p-values between two adjacent loci along bovine chromosomes 2, 6, 14, 19, 21 and 23 affecting more than one growth trait in the M1 commercial line of *Bos taurus* from Beefbooster Inc. Haplotypes were defined by two alleles of a pair of loci. For example, haplotype RM 185-99/BM 1818-267 represented a segment of chromosome having allele 99 of RM 185 and allele 267 of BM 1818. The genetic map distance was indicated in cM. The dashed line represented the comparison-wise threshold level, whereas the solid line represented the chromosome-wise p-value threshold level.







5.4 Future research possibilities

Marker assisted selection (MAS) relies on the selection for specific alleles using genetic markers, detectable genes or DNA fragments that are linked closely enough to loci of interest that they can be used to identify alleles at those loci (Bourdon 2000). The use of MAS, for simply-inherited traits, is common – one gene controls one trait, and thus can be easily selected, or not selected. Marker assisted selection is possible for polygenic, or quantitative, traits, and would be a very useful application in beef cattle breeding systems. The effectiveness of MAS depends on the proximity of the marker to the gene of interest, the ability to determine whether allelic variations at these loci are segregating in a population, and an understanding of the interactions, between genes and between genes and the environment, affecting economic traits. Quantitative trait loci are presumed to have major effects on polygenic traits. Commercial lines of beef cattle are thought to have originated from a few common ancestors. The more closely linked a particular marker and a gene are, the less chance of recombination, and subsequently a greater chance that the two will be passed on together in future generations.

In cattle, linkage disequilibrium (LD) is commonly observed and extends over tens of centimorgans; it has become a widely accepted approach for mapping QTL (Farnir et al. 2000). Co-segregation between a particular genetic marker and quantitative trait loci (QTL) in a population is critical for successfully mapping QTL. Commercial lines of cattle represent semi-closed populations,

typically derived from one or a limited number of founders. Therefore, common haplotypes, harboring QTL of interest, are expected to carry on and segregate among individuals of commercial breeding lines.

In this study, we found that some haplotypes tend to occur more frequently than others; this is probably attributable to a limited number of common ancestors and artificial selection by breeders over many generations. Selection operates to keep certain blocks of genes or genetic material together, thus linkage disequilibrium occurs, especially in populations in which outbreeding is limited and the population originated from a few common founders. Identical-by-descent (IBD) haplotype sharing analysis for QTL mapping relies on linkage disequilibrium (deVries et al. 1996, Riquet et al. 1999, Fallin et al. 2001). A direct haplotype comparison can be used to identify the location of a QTL of interest because the chromosome segment housing the QTL is likely to be segregating in the population. This method of fine mapping QTL is not dependent on a large number of progeny; selection in favor of desirable traits leads to the selection of particular haplotypes thus maintaining linkage disequilibrium. In addition, because haplotypes span two or more loci, both heterozygous and homozygous loci are informative. Therefore, the haplotype sharing method for mapping QTL is both efficient and cost-effective.

Selection based on an index and culling levels described by MacNeil and Newman (1994) was utilized to develop the Beefbooster M1 line. The culling

of calves, or dams and sires producing calves, with minimum or maximum BWT, minimum PWADG, and minimum ADGF, along with selection of individuals for traits within these constraints likely led to the higher frequency of haplotypes harboring genes for lower birth weight, higher pre-weaning average daily gain and higher average daily gain on feed. Given the selection constraints, it would be possible to have an animal with low birth weight and high gain, as well as having an animal with high birth weight and high gain because these were among the traits being selected for.

Haplotypes that are associated with lower birth weight and higher pre- and post-weaning gain would be very appealing to beef cattle producers. Lower birth weight helps to reduce calving difficulties, and subsequently calf morbidity and calf mortality. In addition, an easier calving would be less stressful on the dam and she would be better able to remain in good condition and raise a healthier calf. Higher weight gain means greater returns for the producer, helping to keep him in business.

In order to fit individual environments and producer's operations the identification of genes or genomic regions affecting birth weight and not yearling weight or, conversely, yearling weight and not birth weight, would be powerful tools for influencing pre- and post-weaning growth rates. There is a strong positive correlation between direct effects on birth weight and yearling weight. This indicates that bulls selected for increased post-weaning growth potential may also sire calves having greater birth weight. This results in

increased risk of dystocia. Likewise, breeders seeking to reduce dystocia often do so through the selection of sires with genetic potential to reduce birth weight, thus sacrificing growth potential of the calves. The identification of genes or genomic regions affecting pre- and post-weaning growth coupled with marker-assisted selection has the potential to conquer this genetic antagonism by allowing selection for growth during specific developmental stages. This would help to decrease both the incidence of dystocia and economic loss associated with calving difficulty, while minimizing any concurrent effect on post-weaning growth. Therefore, selection of loci that affect birth weight, but not subsequent growth, would be favorable. With QTLs, it is possible that one QTL can significantly increase weaning weight without concomitantly increasing birth weight (Beever et al. 1990). The discovery of genes affecting birth weight, but not growth at other developmental stages, will prove valuable in more accurately describing the genetic merit of animals to be used for breeding. Coupled with MAS, the exploitation of such loci would provide a mechanism for reducing birth weight and thus the incidence and severity of dystocia without affecting post-weaning growth (MacNeil and Grosz, 2001).

It has been determined that QTLs or genes affecting one trait in one herd, or line, may affect another related trait in another herd, or line (personal communication, ChangXi Li). For example, a QTL may affect birth weight in the M1 line, and the same QTL may affect post-weaning average daily gain on feed in the M3 line. Thus, a QTL affecting growth at one developmental stage

in one population has the ability to affect growth at another developmental stage in a different population. Thus, care must be taken when attempting to integrate a particular QTL or trait into a population, as it may not have the same effect in the new population as was founded in the original population.

Previously, QTLs affecting growth have been localized to a number of bovine chromosomes in reference herd families. Often the QTLs that were identified spanned large chromosomal regions of more than thirty centimorgans. The significance of this project was to verify these previously identified QTLs and narrow down the regions in which they reside. Some regions were narrowed down to less than ten centimorgans, however, there are regions which still span more than twenty centimorgans. It is imperative that these regions be narrowed down further to validate the QTLs identified here. This would be possible, and more effective, if cattle herds were larger and more markers, situated closer together, could be utilized.

Following the research in identifying and fine mapping of QTLs for growth traits on bovine chromosomes 2, 6, 14, 19, 21 and 23 candidate genes were sought out. The candidate genes that were chosen were based on position and function. A variety of sources including the MARC map, the IBRP map, and Womack's RH map were consulted as references to locate possible candidate genes. The candidate genes chosen are displayed in Table 5.

Now that QTLs have been fine mapped and possible candidate genes have been chosen to study, the true candidate genes can be identified and analysis of allelic variation in these genes can be carried out. Isolation of genes will provide a greater understanding of the biology of the gene and its associated traits; eventually favorable alleles can be introgressed into existing herds via breeding. Genomics is a fast developing field in many species. This project represents a step on the path to increasing the resolution and information value of the bovine genome map.

Table 5 List of possible candidate genes for growth traits on BTA 2, 6, 14, 19, 21 and 23 in a commercial line of *Bos taurus*.

BTA	FULL NAME OF POSSIBLE CANDIDATE GENE	ABBREVIATION
BTA 2	growth differentiation factor 8	GDF8
BTA 2	collagen, type III, alpha 3	CoL3A3
BTA 2	NGFI-A binding protein 1	NAB1
BTA 2	eukaryotic translation initiation factor 3, subunit 2	E1F3S2
BTA 2	collagen, type XVI, alpha 1	CoL16A1
BTA 2	myostatin	MSTN
BTA 6	ATP synthase, H ⁺ transporting, mitochondrial FO complex, subunit e	ATP51
BTA 6	replication factor C (activator 1)	RFC1
BTA 6	UDP glucose dehydrogenase	UGDH
BTA 6	LIM protein (similar to rat protein kinase C - binding enigma)	LIM
BTA 6	fibroblast growth factor receptor 3	FGFR3
BTA 6	adducin 1	ADD1
BTA 6	WD repeat domain 1	WDR1
BTA 6	sulfotransferase, estrogen preferring	STE
BTA 6	cathepsin L	CTSL
BTA 6	secreted phosphoprotein 1	SPP1
BTA 14	protein kinase (c-AMP dependent, catalytic) inhibitor alpha	PKIA
BTA 14	fatty acid binding protein 5 (psoriasis associated)	FABP5
BTA 14	carbonic anhydrase III, muscle specific	CA3
BTA 14	collagen, type XIV, alpha 1	CoL14A1
BTA 14	E2F transcription factor 5 p130, binding	E2F5
BTA 14	protein tyrosine kinase 2 beta	PTK2
BTA 14	tissue specific transplantation antigen P35B	TSTA3
BTA 19	collagen, type I, alpha 1	CoLA1
BTA 19	sarcoglycan, alpha	SGCA
BTA 19	pyruvate dehydrogenase kinase, isoenzyme 2	PKD2
BTA 19	integrin, alpha 3	ITGA3
BTA 21	cellular retinoic acid binding protein	CRABP1
BTA 21	isocitrate dehydrogenase 2 (NADP ⁺), mitochondrial	IDH2
BTA 21	milk fat globule - EGF factor 8 protein	MFGE8
BTA 21	isocitrate dehydrogenase 3 (NAD ⁺) alpha	IDH3A
BTA 23	aldehyde dehydrogenase 5 family member A1 (succinate semialdehyde dehydrogenase)	ALDH5A1
BTA 23	MyoD family inhibitor	MDF1
BTA 23	vascular endothelial growth factor	VEGF
BTA 23	protein tyrosine kinase 7	PTK7
BTA 23	suppressor of Ty(S.cerevisiae) 3 homolog	SUPT3H
BTA 23	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	AGPAT1
BTA 23	collagen, type XI, alpha 2	CoL11A2

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7. APPENDIX

Table 7.1	Bovine chromosome 2 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.2	Bovine chromosome 6 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.3	Bovine chromosome 14 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.4	Bovine chromosome 19 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.5	Bovine chromosome 21 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.6	Bovine chromosome 23 genetic markers with sequence, size, number of alleles and marker type data.
Table 7.7	Frequencies for common haplotypes analyzed on bovine chromosome 2, 6, 14, 19, 21 and 23.

Table 7.1 Bovine chromosome 2 markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Size Range (bp)	# Alleles	Marker type
TGLA 44	AACTGTATATT GAGAGCCTACCATG	CACAACCTTAGCG ACTAAACCACCA	136 - 172	16	microsatellite
TGLA 431	GGTCATATCCTT CAAAATTTACTTA	CCCATTATTTCCT AATTTCAACTTC	134 - 163	9	microsatellite
TEXAN 2	ACATTGTCATG TGGTTGCTAAC	ACTCTGGGTATGT ATATGTGCAAG	106 - 128	10	microsatellite
CSSM 042	GGGAAGGTCCTA ACTATGGTTGAG	ACCCTCACTTCT AACTGCATTGGA	175 - 219	10	microsatellite
BMS 1300	TCCTGGGCCTGG TAAATAT	CTTTGGTTTGG AAAGAAGTTG	153 - 171	7	microsatellite
ILSTS 098	AGGAATCACT GGATAGATGC	AGTGTATACT GCTTCTCCC	97 - 123	10	microsatellite
BMS 1837	ACGATCTAGTAA TAGTTGTTTACA	TTCATTATTCCTT CCTTTGCTTC	83 - 109	7	microsatellite
BMS 1866	CAGGGACTGAA AAATAATGCC	TTCCATGTTGA TTGTTTCTTCC	134 - 158	11	microsatellite
TEXAN 4	AGAAGTCCTTGC TTTCACAGAC	TTTTGGTGCTT GATGCTATTAG	110 - 136	7	microsatellite
TEXAN 5	TGGAAATACCG ACTCCTGATTC	CATGGTCTAACAT CTAATAAATGCC	138 - 150	3	microsatellite
BMS 356	ACCTCAGAGA TGACGCAAGG	TTGAAGTTTTT GTGCTGTTTGG	88 - 104	5	microsatellite
BM 2113	GCTGCCTTCT ACCAAATACCC	CTTCCTGAGAG AAGCAACACC	123 - 143	11	microsatellite
OARFCB 11	GCAAGCAGGTTCT TTACCACTAGCACC	GGCCTGAACCTACAAG TTGATATATCTATCAC	139 - 168	14	microsatellite

Table 7.2 Bovine chromosome 6 genetic markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Size Range (bp)	# Alleles	Marker type
INRA 133	ATCCTCAAAG CAACCTGGC	GAATCTTCTCC CCCTGCATC	206 - 232	7	microsatellite
ILSTS 090	TAGTACCATAC CCCAGGTAGG	GCCAAAACAC ACAAGTGTGC	143 - 147	3	microsatellite
BM 1329	TTGTTTAGGCAA GTCCAAAGTC	AACACCGCA GCTTCATCC	145 - 161	8	microsatellite
BMS 2508	TTTCTGGGATT ACAAAATGCTC	TTTCTTAGGGGA GTGTTGATTG	87 - 111	9	microsatellite
BMS 382	GGCACATATGA ATAAATGCTTTG	TCTGACACAACCTT AGCAACTAAACA	158 - 178	7	microsatellite
BMS 1242	AGTGTGATCAA CAACGGCAG	AGTACTGGTG CAGTGCTTG	98 - 126	10	microsatellite
BM 4322	TATCACCACC ATGGCAAATG	GACTCACCATA CTCCAGAGCC	165 - 187	6	microsatellite
BMS 470	TAAAATGTGC TCACTACCTGGA	CAAATATGAAGG TGTGGGACTC	59 - 85	9	microsatellite
BMS 360	ACAAAACCAC TTTCTTAGCAAACA	CTGGGTCTTC ATGGTAGGGA	74 - 102	11	microsatellite
BM 4528	CAGAATCCATA CACATGTCAACA	AGGAACAGGTAT AGGAATATTGGA	238 - 276	8	microsatellite
RM 028	CTACAGTCATG GGTCTGAAAGAG	TACCCGGGGATCTTC AGCCTGGCCTGAGAG	94 - 110	5	microsatellite
OAREL 03	GCTTCCTTGAAA GGAAAGCAGTC	AGACAAGTTCTG CAGATACCCTGC	147 - 163	8	microsatellite
ILSTS 087	AGCAGACATG ATGACTCAGC	CTGCCTCTTT TCTTGAGAGC	117 - 125	5	microsatellite
BM1236	CTGAACCAC CACGGAAGC	AGTCCATGG GGTTGCAAG	116 - 128	7	microsatellite
BMS 2460	TGGACTGTCA AACGTAGGGT	CAAGTCAGGAAA GATGAAACTTG	67 - 83	7	microsatellite
ETH 8	GGCGAGGCAGG TGAGATGCCAA	CAGAGCCTACAC AGTGCACACCA	306 - 330	5	microsatellite
BMC 4203	GCAAATGTAA GCTGAAGGCC	CCTGGGAAAT CCCATGGAC	144 - 170	10	microsatellite

Table7.3 Bovine chromosome 14 genetic markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Min. Size (bp)	# Alleles	Marker type
DGAT 1	CGCTTGCTC GTAGCTTTGG	CCGCGGTAG GTCAGGTTGT	83	2	SNP
CSSM 66	ACACAAATCCTT TCTGCCAGCTGA	AATTTAATGCAC TGAGGAGCTTGG	179 - 199	11	microsatellite
BMS 1747	TCTAAGCTCCTT GAAGACAGGC	GGCTTTGTATT CCCCTCTCC	81 - 99	9	microsatellite
TG	GGGGATGACTAC GAGTATGACTG	GTGAAAATCTTG TGGAGGCTGTA	545	2	RFLP
BMS 1678	TCTTCTCTGCA CTTTGGTTGC	ATAGCTGACA TCCACTGGGC	123 - 135	7	microsatellite
BMS 1941	TTCTAAATACTC TGAGGTGCAACA	AGCTTATAGTGC TGTACGAAGGTT	112 - 128	7	microsatellite
BMC 1207	ACCAACAAGT CTGAATCTTCATT	GGGTGGAAT AGTCAGTCCCA	128 - 150	10	microsatellite
BM 1577	AGGAGCAGAA CCACTAGGAGG	TGGACTTTAGGC TTGTTTAGCC	140 - 150	5	microsatellite
BMS 108	GGCAATTACAG GATAGAAAGTTG	CTGCCTTCTT TCACTCAACC	92 - 114	10	microsatellite
BMS 1899	GGAGTCAGACAT GACAAGTGAC	TCCTATTCATCC TGTTATTGCC	108 - 128	10	microsatellite
RM 137	ATTGGAATATGA GCTGAGAGTAGG	TCTAAAAAGCCT TATGTAAGTAAGG	147 - 151	3	microsatellite

Table 7.4 Bovine chromosome 19 genetic markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Size Range (bp)	# Alleles	Marker type
BM 6000	ACAGCAATG CCATGGACC	TGCCATTTG GATGTGTGC	106 - 118	5	microsatellite
BMS 745	TAGGGACTTG TTACCCGTGG	TGCAAGCTGT GAGGAGGAG	99 - 121	11	microsatellite
CSSME 70	ATACAGATTA AATACCCACCTG	TTCTAACAGCT GTCCTCAGGC	137 - 145	4	microsatellite
RM 222	CCTAGAGAATTC AATGGACTGAAG	CACAAAGTCACT TTTGTCGGCAA	114 - 136	10	microsatellite
BP 20	TCTGTGGGTG AACAAGCAAG	GGCTCCCTAA AGACCCACTC	219 - 235	9	microsatellite
ILSTS 014	CTGACTATGG TGATAATCCC	TCTTTTCCCT TTCCTTCCCC	126 - 130	3	microsatellite
BMS 2503	TTGAACAATC ACCAGCTTCCC	GACATGACT GAGCGCGTG	147 - 165	5	microsatellite
BM 2389	AATGTTAGGTT TACATGCAGCC	AGGCAATAGGA TCTCCACTAGC	78 - 112	9	microsatellite
BM 17132	ATCTGCCAGTA TCACATCAACA	GTTACTTTTCC AGGCATGAAGC	80 - 104	10	microsatellite
RM 099	CCAAAGAGTCT AACACAACCTGAG	ATCCGAACCAA AATCCCATCAAG	119 - 125	3	microsatellite
CSSM 65	TTCCTGCTTGGT GAAACTTTGAAC	CAACTCAAAGC TTCAACAGCAGCC	154 - 174	9	microsatellite
BMS 1069	GCCCGTACC CAGAAAACC	GGACCAGACT TCACAGGGAG	93 - 113	6	microsatellite
RM 388	GGGGACCATC ACCGTACACTC	GGGACAGCCA GTCTTCTCAG	134 - 150	7	microsatellite
BMS 601	CACTAGGACG ATGCTCTCAGG	TCACAAGAGC AATGACGAGG	175 - 187	8	microsatellite

Table 7.5 Bovine chromosome 21 genetic markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Size Range (bp)	# Alleles	Marker type
BM 8115	AATCAACATCC AGATTTCTTTTG	CCACCCCAAA GACCTTTCTA	119 - 143	12	microsatellite
BMS1117	TGTGTGCTCTC TCACACATGC	AACCAAAGC AGGGATCAGG	83 - 103	9	microsatellite
AGLA 233	TGCAAACATCCA CGTAGCATAAATA	GCATGAACAGCC AATAGTGTTCATC	242 - 250	6	microsatellite
BP 33	AGGCCTCTG AATTCCTCTCC	AACCAGCAGT CTTGCTCTCTC	253 - 283	10	microsatellite
BMS 2815	TGATATTCAAA CTCAATGAACCC	CTTG CATATGC TCATCATTATCA	83 - 115	10	microsatellite
ILSTS 092	GAGAAACTT TGGGCTGCTGC	ATGGATTGCT TCTGTGGACC	173 - 181	4	microsatellite
ILSTS 054	GAGGATCTTGA TTTTGATGTCC	AGGGCCACTA TGGTACTTCC	126 - 150	9	microsatellite
BMS 2382	AGCACGGAG TCGTTGTCTG	CCATCTGGA CAGAACGTTACC	183 - 195	5	microsatellite

Table 7.6 Bovine chromosome 23 genetic markers with sequence, size, number of alleles and marker type data.

Marker	Forward Sequence	Reverse Sequence	Size Range (bp)	# Alleles	Marker type
INRA 064	AACATTTCA GCTGATGGTGGC	TTCTGTTTTGA GTGGTAAGCTG	177 - 187	10	microsatellite
RM 033	GCTCATCTCC TGGGATGCAGA	GCTCCTTTAGTT TTCTTGTTGGAG	150 - 166	8	microsatellite
BM 1815	AGAGGATGA TGGCCTCCTG	CAAGGAGACAA GTCAAGTTCCC	140 - 170	8	microsatellite
BM 1258	GTATGTATTT TTCCCACCCTGC	GAGTCAGACAT GACTGAGCCTG	100 - 128	9	microsatellite
BMS 468	GTTAAGCAG AGGGTTTCCCC	TATTCCCAG GTGCTCTGAGG	128 - 134	4	microsatellite
RM 185	TGGCCTGCTT ATGCTTGCATC	GAGTTTCCTTT GCATGCCAGTC	96 - 112	8	microsatellite
BM 1818	AGCTGGGAAT ATAACCAAAGG	AGTGCTTTCA AGGTCCATGC	258 - 272	7	microsatellite
CSSM 24	GAGGATGCAGA ACCAGGCGTTAGA	GGTCGAAGAGA GTCAGACATGACT	103 - 223	12	microsatellite
BM 144	AAATAAAGAGA CATGGTCACCGG	TCGAGGTGT GGGAGGAAG	137 - 165	11	microsatellite

Table 7.7 Frequencies for common haplotypes analyzed on bovine
chromosome 2, 6, 14, 19, 21 and 23.

HAPLOTYPE	BTA	FREQUENCY (%)
TGLA 44-162/TGLA 431-141	2	9.09%
TGLA 44-162/TGLA 431-146	2	11.36%
TGLA 44-166/TGLA 431-141	2	13.07%
TGLA 44-166/TGLA 431-146	2	13.64%
TGLA 431-141/TEXAN 2-116	2	27.27%
TGLA 431-146/TEXAN 2-116	2	30.68%
TGLA 431-165/TEXAN 2-116	2	10.23%
TEXAN 2-116/CSSM 042-182	2	31.82%
TEXAN 2-116/CSSM 042-207	2	8.52%
TEXAN 2-116/CSSM 042-216	2	19.32%
CSSM 042-182/BMS 1300-164	2	15.91%
CSSM 042-216/BMS 1300-164	2	9.66%
BMS 1300-164/ILSTS 098-97	2	11.93%
ILSTS 098-97/BMS 1837-88	2	18.44%
BMS 1837-88/BMS 1866-135	2	31.25%
BMS 1837-88/BMS 1866-141	2	11.93%
TEXAN 4-133/TEXAN 5-147	2	8.52%
TEXAN 4-127/TEXAN 5-147	2	13.64%
TEXAN 5-147/BMS 356-99	2	41.48%
TEXAN 5-147/BMS 356-101	2	23.30%
BMS 356-99/BM 2113-128	2	18.75%
BMS 356-99/BM 2113-137	2	8.52%
BMS 356-99/BM 2113-139	2	12.50%
BMS 356-99/BM 2113-143	2	11.36%
BM 2113-128/OARFCB 11-161	2	9.66%
BM 2113-143/OARFCB 11-161	2	17.05%
BM 2113-139/OARFCB 11-161	2	15.91%
INRA 133-208/ILSTS 090-149	6	14.20%
INRA 133-218/ILSTS 090-149	6	11.36%
INRA 133-220/ILSTS 090-149	6	13.64%
ILSTS 090-149/BM 1329-2	6	17.05%

ILSTS 090-149/BM 1329-3	6	13.64%
ILSTS 090-149/BM 1329-5	6	46.59%
BM 1329-5/BMS 2508-106	6	13.07%
BM 1329-5/BMS 2508-104	6	10.80%
BM 1329-5/BMS 2508-102	6	15.34%
BM 1329-3/BMS 2508-104	6	9.09%
BMS 2508-102/BMS 382-166	6	13.64%
BMS 382-168/BM 1242-100	6	
BM 4322-2/BMS 470-68	6	13.64%
BM 4322-5/BMS 470-68	6	8.52%
BMS 470-60/BMS 360-8	6	9.66%
BMS 470-68/BMS 360-8	6	14.20%
BMS 360-5/BM 4528-2	6	12.50%
BMS 360-8/BM 4528-1	6	11.93%
BMS 360-8/BM 4528-2	6	36.36%
BM 4528-2/BM 4621-139	6	15.91%
BM 4528-2/BM 4621-143	6	39.20%
BM 4528-3/BM 4621-143	6	12.50%
BM 4621-143/RM 028-3	6	18.18%
BM 4621-143/RM 028-2	6	17.05%
RM 028-2/OAREL 03-2	6	32.95%
OAREL 03-2/ILSTS 087-2	6	32.95%
OAREL 03-3/ILSTS 087-2	6	18.18%
OAREL 03-4/ILSTS 087-2	6	17.61%
OAREL 03-5/ILSTS 087-2	6	32.95%
ILSTS 087-2/BM 1236-118	6	53.98%
ILSTS 087-2/BM 1236-122	6	29.55%
ILSTS 087-2/BM 1236-126	6	15.91%
BM 1236-118/BMS 2460-3	6	17.61%
BM 1236-118/BMS 2460-5	6	25.57%
BM 1236-122/BMS 2460-4	6	67.61%
BM 1236-122/BMS 2460-5	6	9.09%
BMS 2460-5/ETH 8-331	6	10.80%
BMS 2460-5/ETH 8-333	6	9.66%
ETH 8-331/BMC 2403	6	9.66%

DGAT 1-9/CSSM 66-1	14	30.11%
DGAT 1-9/CSSM 66-6	14	15.91%
DGAT 1-9/CSSM 66-7	14	31.25%
DGAT 1-9/CSSM 66-9	14	31.82%
CSSM 66-1/BMS 1747-3	14	18.18%
CSSM 66-1/BMS 1747-8	14	10.23%
BMS 1747-3/TG -2	14	19.32%
BMS 1747-3/TG -3	14	17.05%
BMS 1747-8/TG -2	14	28.41%
TG -2/BMS 1678-4	14	12.50%
TG -2/BMS 1678-5	14	29.07%
TG -2/BMS 1678-6	14	46.02%
TG -3/BMS 1678-4	14	9.66%
BMS 1678-4/BMS 1941-10	14	18.75%
BMS 1678-5/BMS 1941-10	14	18.75%
BMS 1678-6/BMS 1941-10	14	18.18%
BMS 1941-4/BMC 1207-3	14	10.80%
BMS 1941-10/BMC 1207-2	14	15.34%
BMS 1941-10/BMC 1207-3	14	26.14%
BMS 1941-10/BMC 1207-12	14	11.36%
BMC 1207-2/BM 1577-1	14	26.70%
BMC 1207-3/BM 1577-1	14	31.25%
BMC 1207-12/BM 1577-1	14	10.23%
BMC 1207-12/BM 1577-5	14	10.80%
BM 1577-1/BMS 108-3	14	17.05%
BM 1577-1/BMS 108-4	14	19.32%
BM 1577-1/BMS 108-6	14	39.77%
BMS 108-4/BMS 1899-6	14	9.66%
BMS 108-6/BMS 1899-12	14	11.36%
BMS 1899-2/RM 137-2	14	9.66%
BMS 1899-3/RM 137-2	14	12.50%
BMS 1899-6/RM 137-2	14	30.68%
BMS 1899-8/RM 137-2	14	15.34%
BM 6000-6/BMS 745-2	19	13.64%
BM 6000-6/BMS 745-4	19	19.32%
BM 6000-6/BMS 745-6	19	21.59%
BM 6000-6/BMS 745-7	19	25.57%

BMS 745-7/CSSME 70-3	19	10.80%
CSSME 70-3/RM 222-0	19	8.52%
BP 20-2/ILSTS 014-3	19	20.45%
BP 20-3/ILSTS 014-3	19	38.07%
BP 20-4/ILSTS 014-3	19	13.07%
ILSTS 014-3/BMS 2503-158	19	27.84%
ILSTS 014-3/BMS 2503-164	19	31.25%
BMS 2503-158/BMS 2389-2	19	15.91%
BMS 2503-158/BMS 2389-8	19	10.80%
BMS 2503-164/BMS 2389-5	19	14.20%
BMS 2503-164/BMS 2389-9	19	11.93%
BMS 2389-2/BM 17132-86	19	12.50%
BM 17132-86/RM 099-126	19	19.32%
BM 17132-86/RM 099-128	19	11.36%
BM 17132-88/RM 099-126	19	11.93%
BM 17132-88/RM 099-128	19	10.23%
RM 099-126/CSSM 65-1	19	17.61%
RM 099-126/CSSM 65-2	19	19.89%
RM 099-128/CSSM 65-2	19	28.41%
CSSM 65-1/BMS 1069-3	19	14.77%
CSSM 65-2/BMS 1069-3	19	26.70%
BMS 1069-3/RM 388-1	19	10.23%
BMS 1069-3/RM 388-2	19	10.80%
BMS 1069-3/RM 388-4	19	25.57%
RM 388-1/BMS 601-5	19	9.09%
RM 388-4/BMS 601-3	19	17.61%
RM 388-4/BMS 601-5	19	27.27%
BM 8115-131/BMS 1117-97	21	13.64%
BM 8115-131/BMS 1117-99	21	10.23%
BMS 1117-95/AGLA 233-253	21	14.20%
BMS 1117-97/AGLA 233-253	21	14.77%
BMS 1117-99/AGLA 233-253	21	9.66%
AGLA 233-253/BP 33-259	21	11.36%
AGLA 233-253/BP 33-269	21	11.36%
AGLA 233-253/BP 33-271	21	9.09%

BP 33-259/BMS 2815-95	21	11.93%
BP 33-263/BMS 2815-95	21	11.36%
BP 33-269/BMS 2815-93	21	10.23%
BMS 2815-93/ILSTS 092-174	21	18.75%
BMS 2815-93/ILSTS 092-176	21	23.86%
BMS 2815-95/ILSTS 092-174	21	33.52%
BMS 2815-95/ILSTS 092-176	21	14.20%
BMS 2815-99/ILSTS 092-174	21	10.23%
ILSTS 092-174/ILSTS 054-6	21	18.18%
ILSTS 092-174/ILSTS 054-9	21	17.61%
ILSTS 092-176/ILSTS 054-6	21	11.93%
ILSTS 092-176/ILSTS 054-9	21	8.52%
ILSTS 054-6/BMS 2382-185	21	22.16%
ILSTS 054-9/BMS 2382-185	21	21.02%
INRA 064-160/RM 033-152	23	35.80%
INRA 064-164/RM 033-152	23	9.09%
RM 033-152/BM 1815-149	23	31.82%
RM 033-152/BM 1815-151	23	9.66%
RM 033-152/BM 1815-163	23	8.52%
RM 033-152/BM 1815-167	23	10.23%
BM 1815-149/BM 1258-103	23	9.66%
BM 1815-149/BM 1258-107	23	12.50%
BM 1258-103/BMS 468-125	23	8.52%
BM 1258-105/BMS 468-125	23	9.66%
BM 1258-107/BMS 468-125	23	11.36%
BMS 468-125/RM 185-99	23	21.59%
RM 185-99/BM 1818-263	23	10.23%
RM 185-99/BM 1818-267	23	14.20%
RM 185-103/BM 1818-263	23	14.77%
BM 1818-263/CSSM 24-228	23	12.50%
BM 1818-267/CSSM 24-226	23	9.09%
BM 1818-267/CSSM 24-228	23	9.09%
CSSM 24-212/BM 1443-156	23	13.64%
CSSM 24-228/BM 1443-154	23	10.80%
CSSM 24-228/BM 1443-156	23	20.45%

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